



US008501926B2

(12) **United States Patent**
Kumar et al.

(10) **Patent No.:** **US 8,501,926 B2**
(45) **Date of Patent:** **Aug. 6, 2013**

(54) **MALARIA VACCINE**

(75) Inventors: **Nirbhay Kumar**, Potomac, MD (US);
Evelina Angov, Silver Spring, MD (US)

(73) Assignees: **The Johns Hopkins University**,
Baltimore, MD (US); **The United States**
of America, as represented by the
Secretary of the Army, Washington, DC
(US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/120,784**

(22) PCT Filed: **Jul. 22, 2009**

(86) PCT No.: **PCT/US2009/004243**

§ 371 (c)(1),
(2), (4) Date: **Mar. 24, 2011**

(87) PCT Pub. No.: **WO2010/036293**

PCT Pub. Date: **Apr. 1, 2010**

(65) **Prior Publication Data**

US 2011/0171266 A1 Jul. 14, 2011

Related U.S. Application Data

(60) Provisional application No. 61/099,651, filed on Sep.
24, 2008.

(51) **Int. Cl.**
A61K 39/015 (2006.01)

(52) **U.S. Cl.**
USPC **536/23.7**; 435/243; 435/320.1; 435/69.3;
424/272.1

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,502,168 A * 3/1996 Kumar 530/388.4
5,753,238 A 5/1998 Kaslow et al.
5,798,106 A * 8/1998 Schoenmakers et al. .. 424/272.1
6,316,000 B1 * 11/2001 Williamson et al. 424/191.1
6,333,406 B1 * 12/2001 Inselburg et al. 536/23.7
6,544,518 B1 * 4/2003 Friede et al. 424/184.1
6,846,481 B1 * 1/2005 Gaertig et al. 424/93.1

(Continued)

FOREIGN PATENT DOCUMENTS

WO 94/12640 * 6/1994
WO 03085114 A1 10/2003

(Continued)

OTHER PUBLICATIONS

Milek, RLB et al, Vaccine, vol. 18, pp. 1402-1411, 2000, Assembly
and expression of a synthetic gene encoding the antigen Pfs48/45 of
the human malaria parasite *Plasmodium falciparum* in yeast.*

(Continued)

Primary Examiner — Albert Navarro

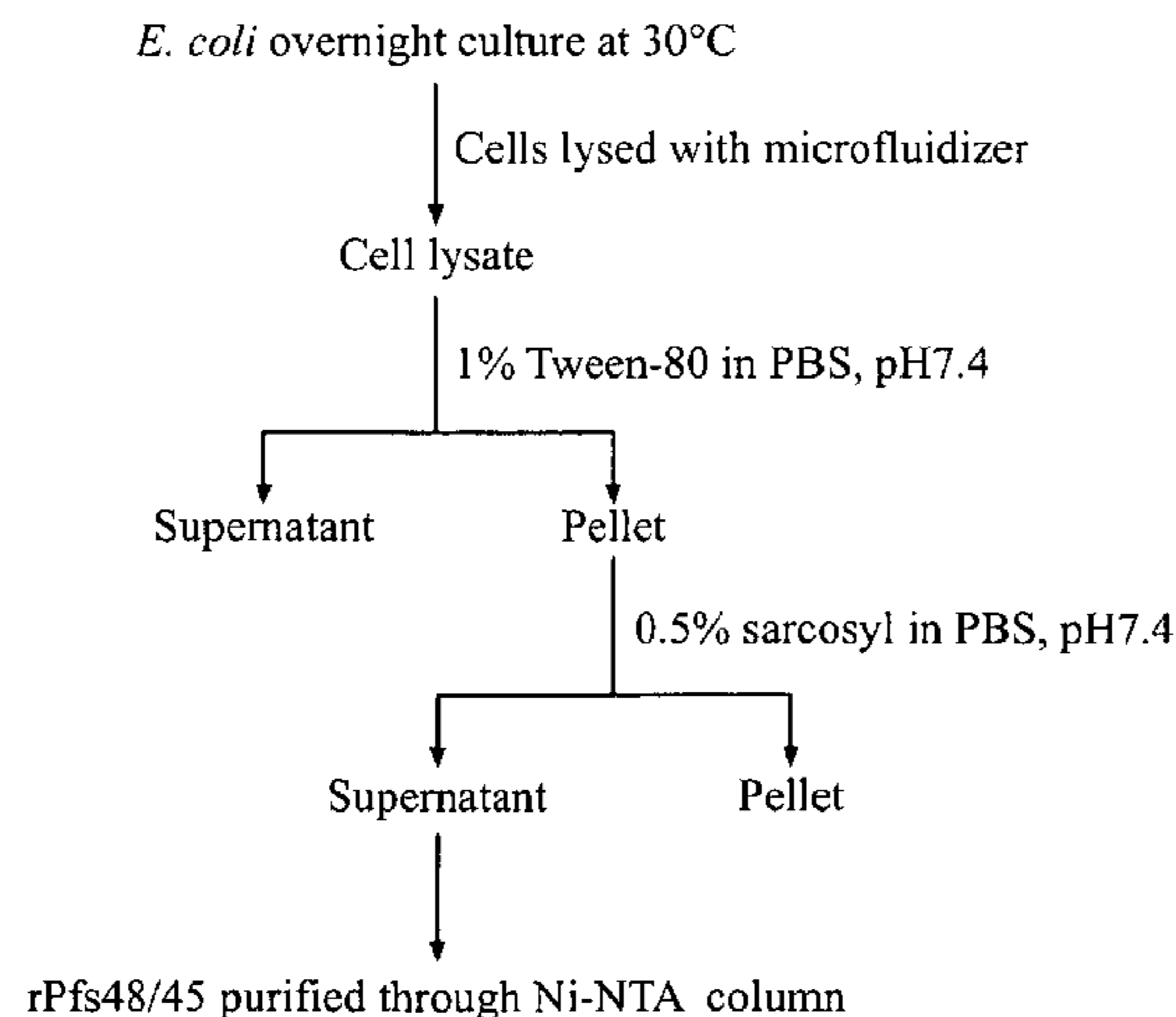
Assistant Examiner — Ginny Portner

(74) *Attorney, Agent, or Firm* — Johns Hopkins Technology
Transfer

(57) **ABSTRACT**

The present invention features immunogenic compositions
based on pre-fertilization or post-fertilization antigens
expressed in the circulating gametocytes in the peripheral
blood of infected persons or on the malaria parasites' stages of
development in the mosquito midgut including extracellular
male and female gametes, fertilized zygote and ookinete. The
invention also features methods to prevent the transmission of
malaria using the immunogenic compositions of the inven-
tion.

5 Claims, 8 Drawing Sheets



U.S. PATENT DOCUMENTS

6,958,235 B1 * 10/2005 Longacre-Andre
 et al. 435/320.1
 7,192,934 B1 * 3/2007 Kaslow et al. 514/44 R
 7,407,658 B2 8/2008 Kaslow et al.
 7,696,308 B2 * 4/2010 Longacre-Andre et al. .. 530/300
 8,273,357 B2 * 9/2012 Hacoheh et al. 424/193.1
 2003/0049278 A1 3/2003 Kaslow et al.
 2004/0137512 A1 * 7/2004 Horii 435/7.1
 2004/0242517 A1 * 12/2004 Cascalho et al. 514/44
 2005/0095256 A1 * 5/2005 Bujard et al. 424/191.1
 2005/0096288 A1 * 5/2005 Guevara, Jr. 514/44
 2005/0265974 A1 * 12/2005 Pau et al. 424/93.2
 2006/0088547 A1 4/2006 Lanar et al.
 2006/0105347 A1 * 5/2006 Meade et al. 435/6
 2006/0147477 A1 * 7/2006 Cabezon Siliva et al. . 424/277.1
 2006/0233789 A1 * 10/2006 Endo et al. 424/133.1
 2007/0009994 A1 * 1/2007 Horii 435/69.3
 2007/0071726 A1 * 3/2007 Pau et al. 424/93.2
 2007/0269410 A1 * 11/2007 Tucker 424/93.2
 2009/0047303 A1 * 2/2009 Shaw et al. 424/194.1
 2010/0055167 A1 * 3/2010 Zhang et al. 424/450
 2010/0062028 A1 * 3/2010 Cohen et al. 424/272.1

FOREIGN PATENT DOCUMENTS

WO 2004/055187 * 7/2004
 WO 2005/025614 * 3/2005
 WO 2006124712 A2 11/2006

OTHER PUBLICATIONS

Octchkourov, NS et al, PNAS, Mar. 18, 2008, vol. 105(11), pp. 4301-4305, Correctly folded Pfs48/45 protein of *Plasmodium falciparum* elicits malaria transmission-blocking immunity in mice.*
 Escalante, AA et al, Molecular and Biochemical Parasitology, vol. 119(1), pp. 17-22, Polymorphism in the gene encoding the Pfs48/45 antigen of *Plasmodium falciparum*. XI Asembo Bay Cohort Project.*
 Milek et al., "Immunological properties of recombinant proteins of the transmission blocking vaccine candidate, Pfs48/45, of the human malaria parasite *Plasmodium falciparum* produced in *Escherichia coli*", Parasite Immunology, vol. 20, No. 8, pp. 377-385 (1998).

* cited by examiner

[MAHHHHHHPGGSGSGT] N₂₈NDF.....TIDS₄₂₇

FIG. 1A

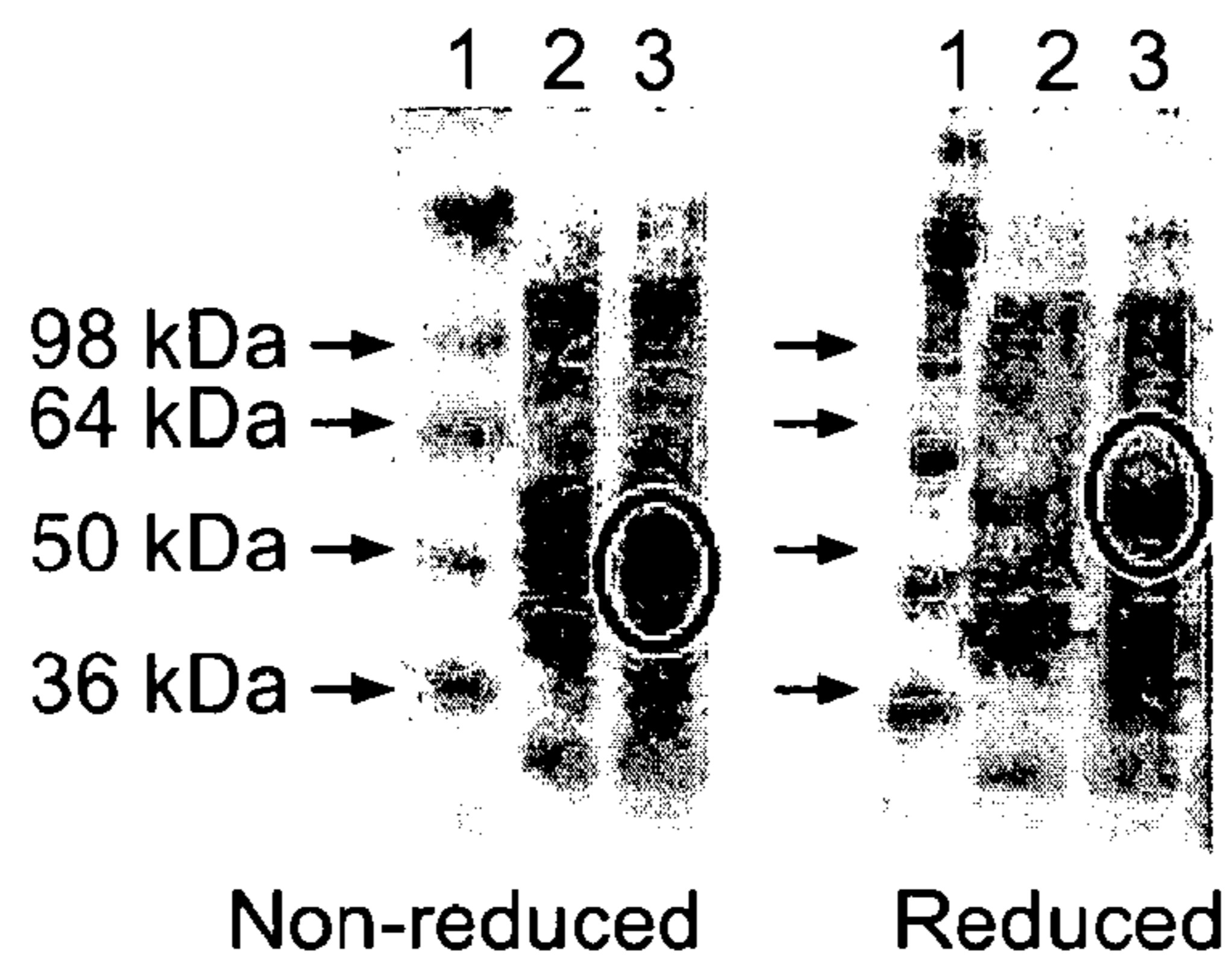


FIG. 1B

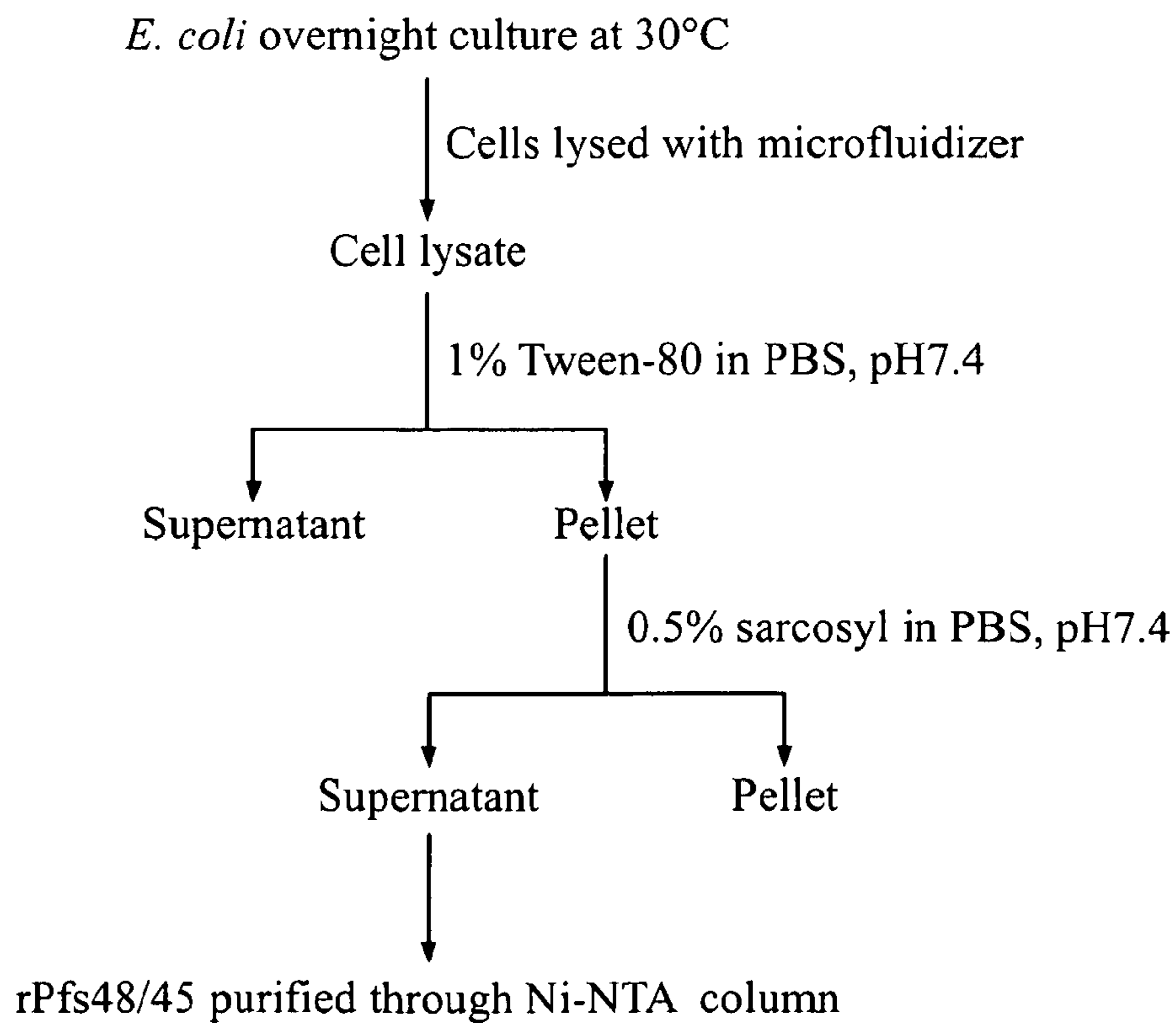


FIG. 1C

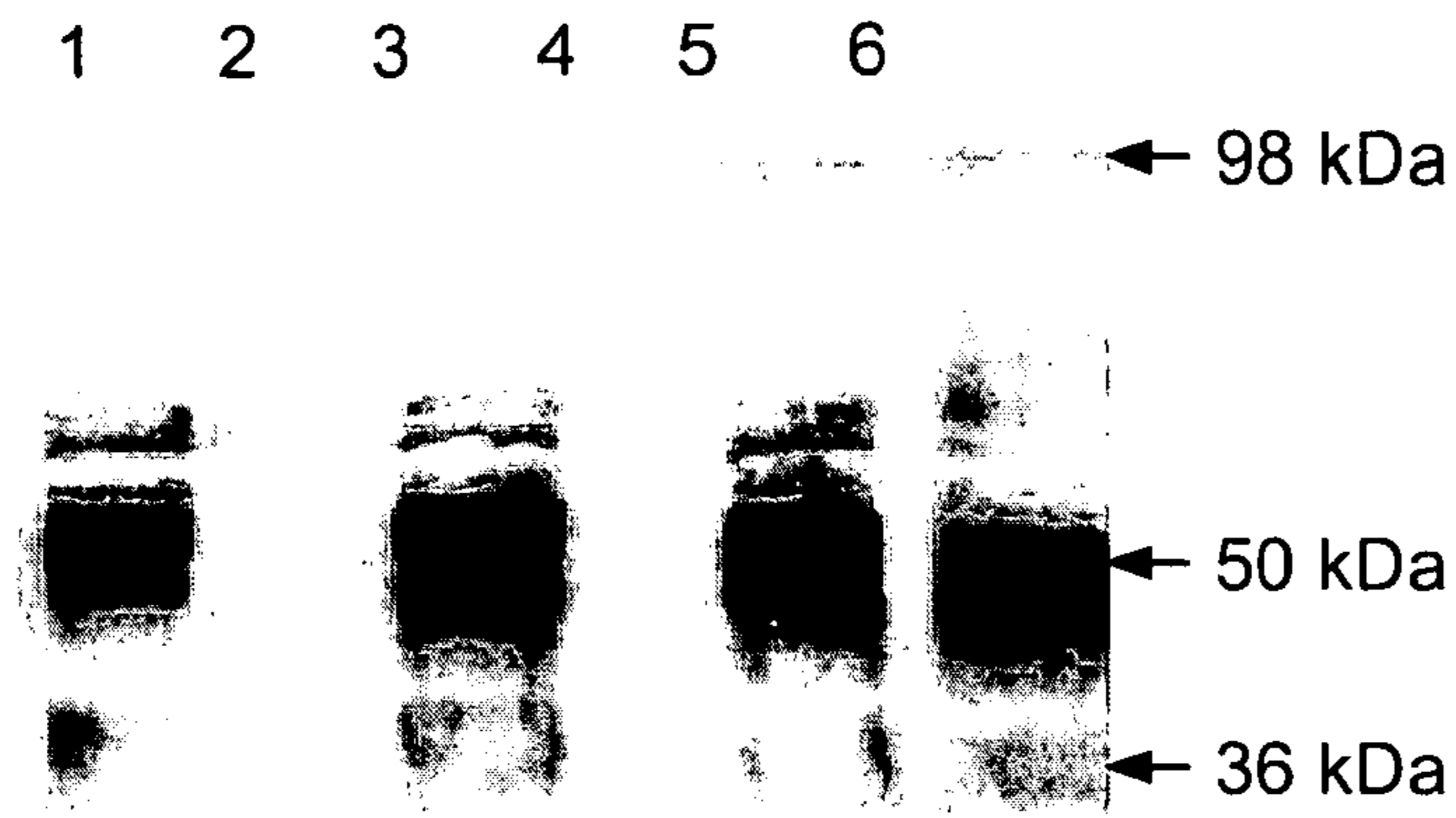


FIG. 1D

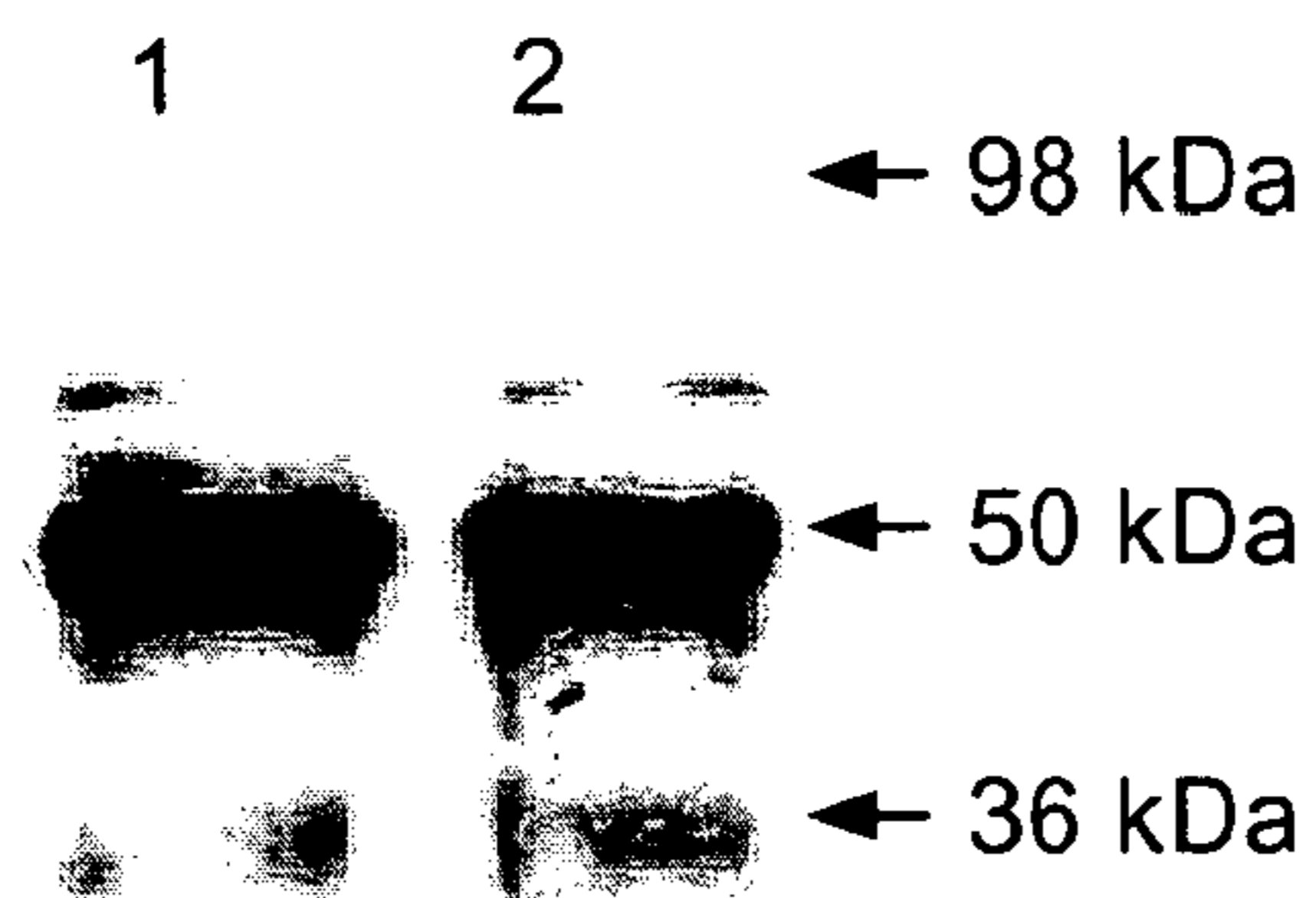


FIG. 1E

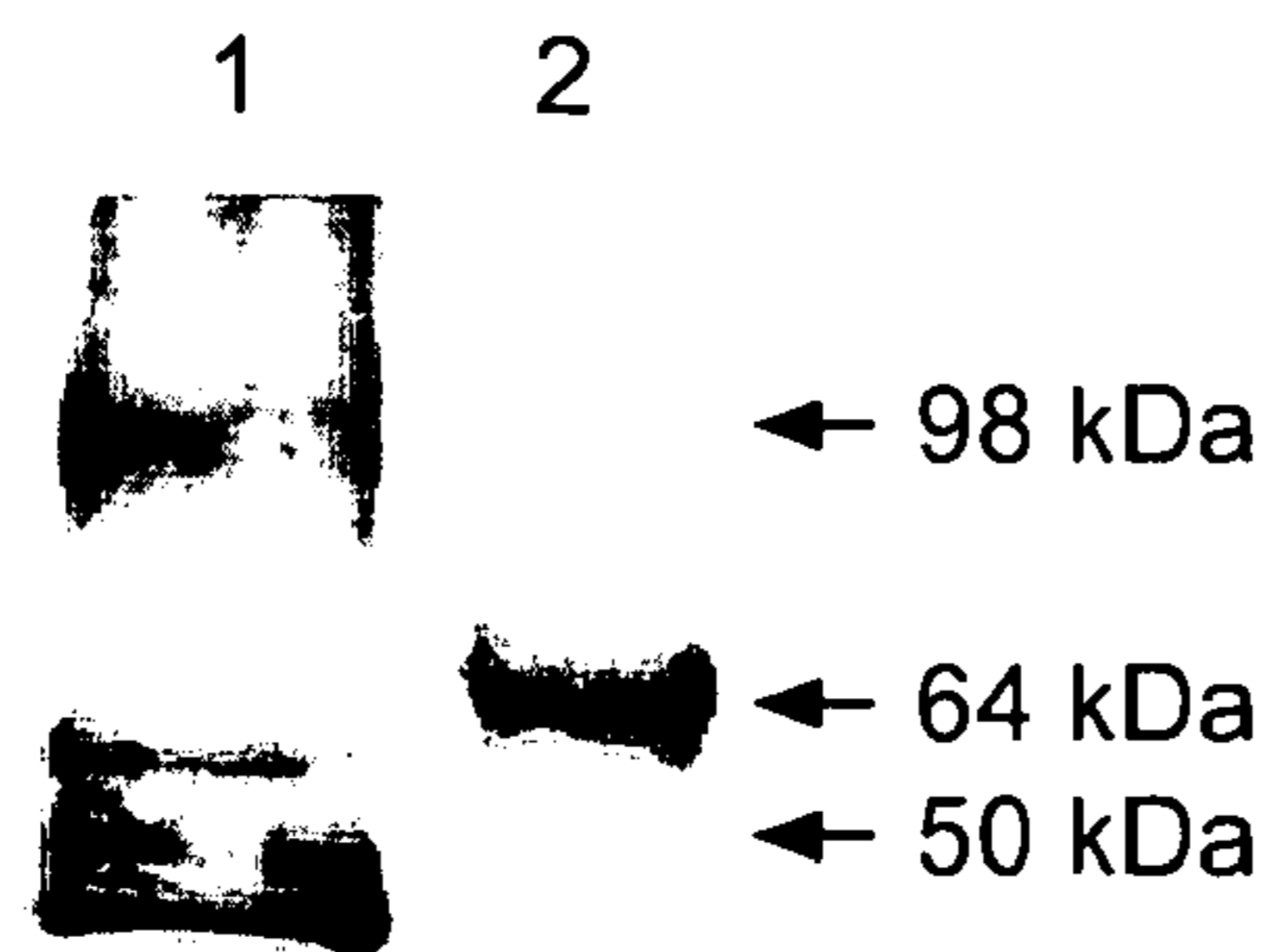


FIG. 1F

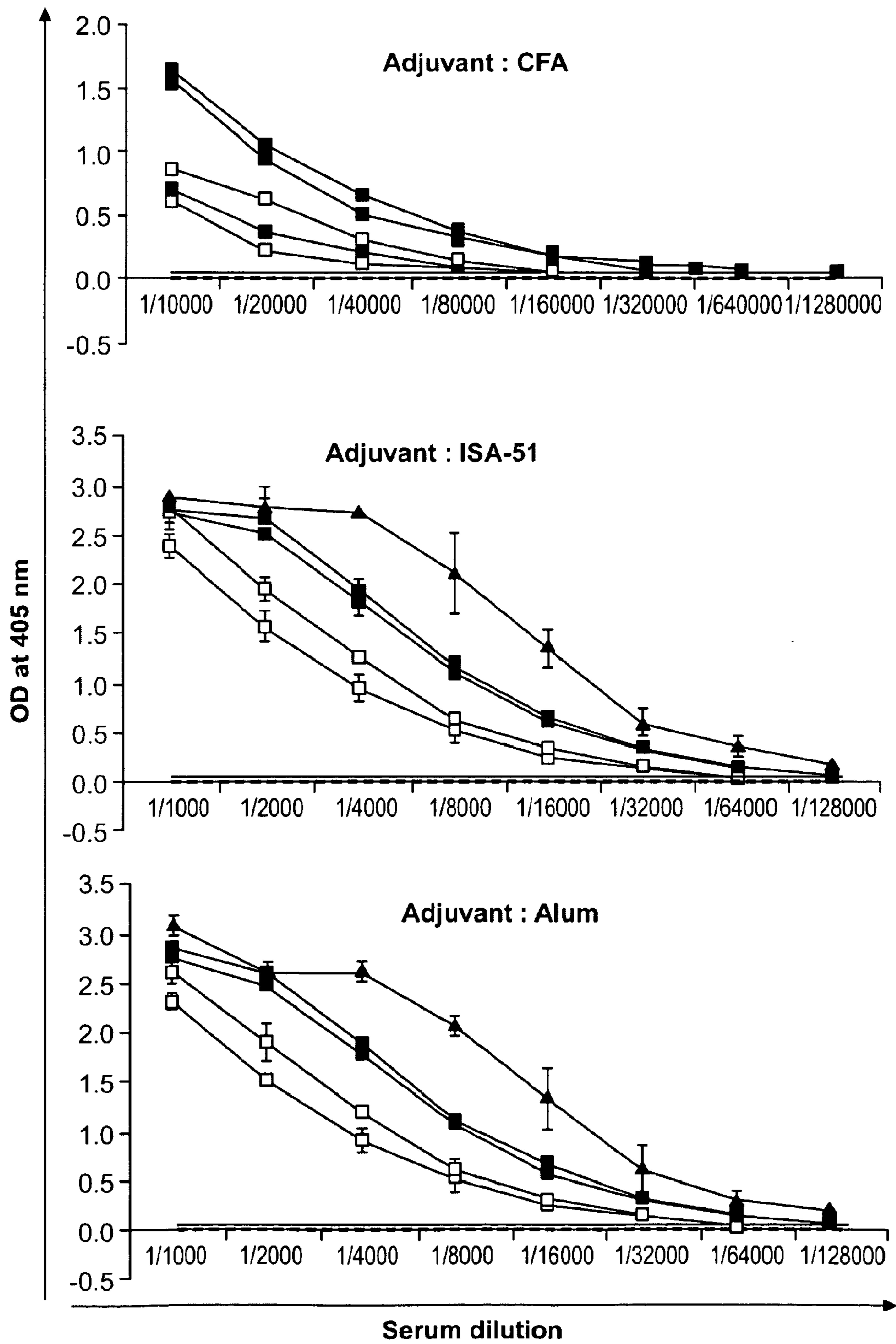


FIG. 2A

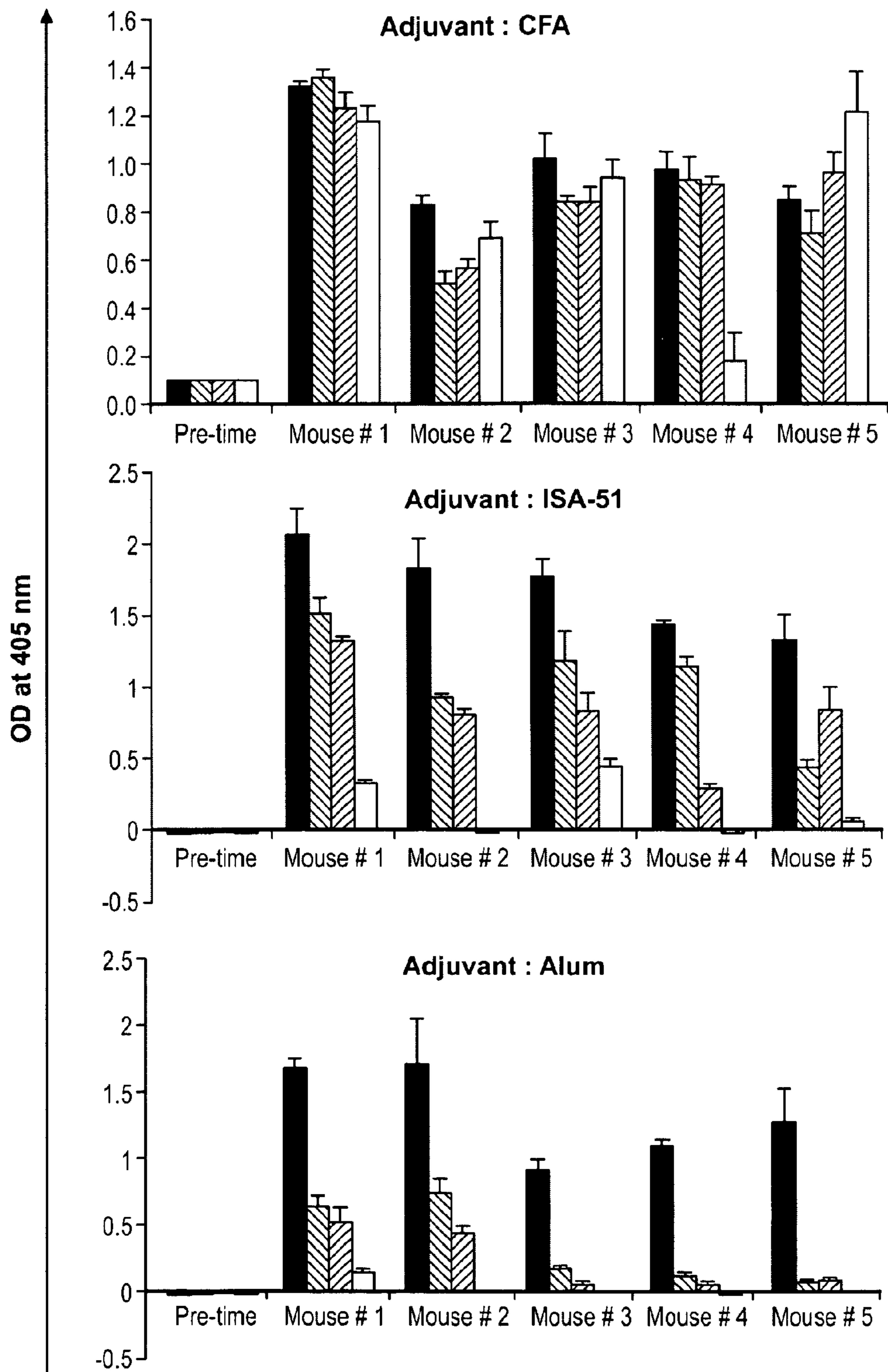


FIG. 2B

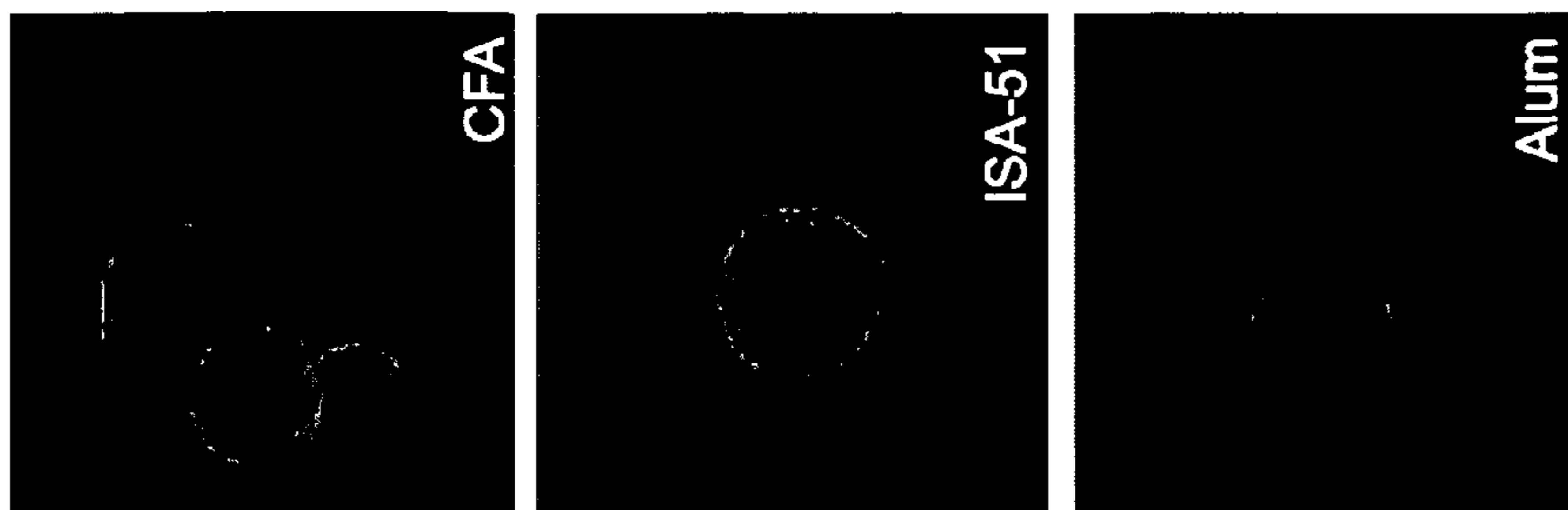


FIG. 3B

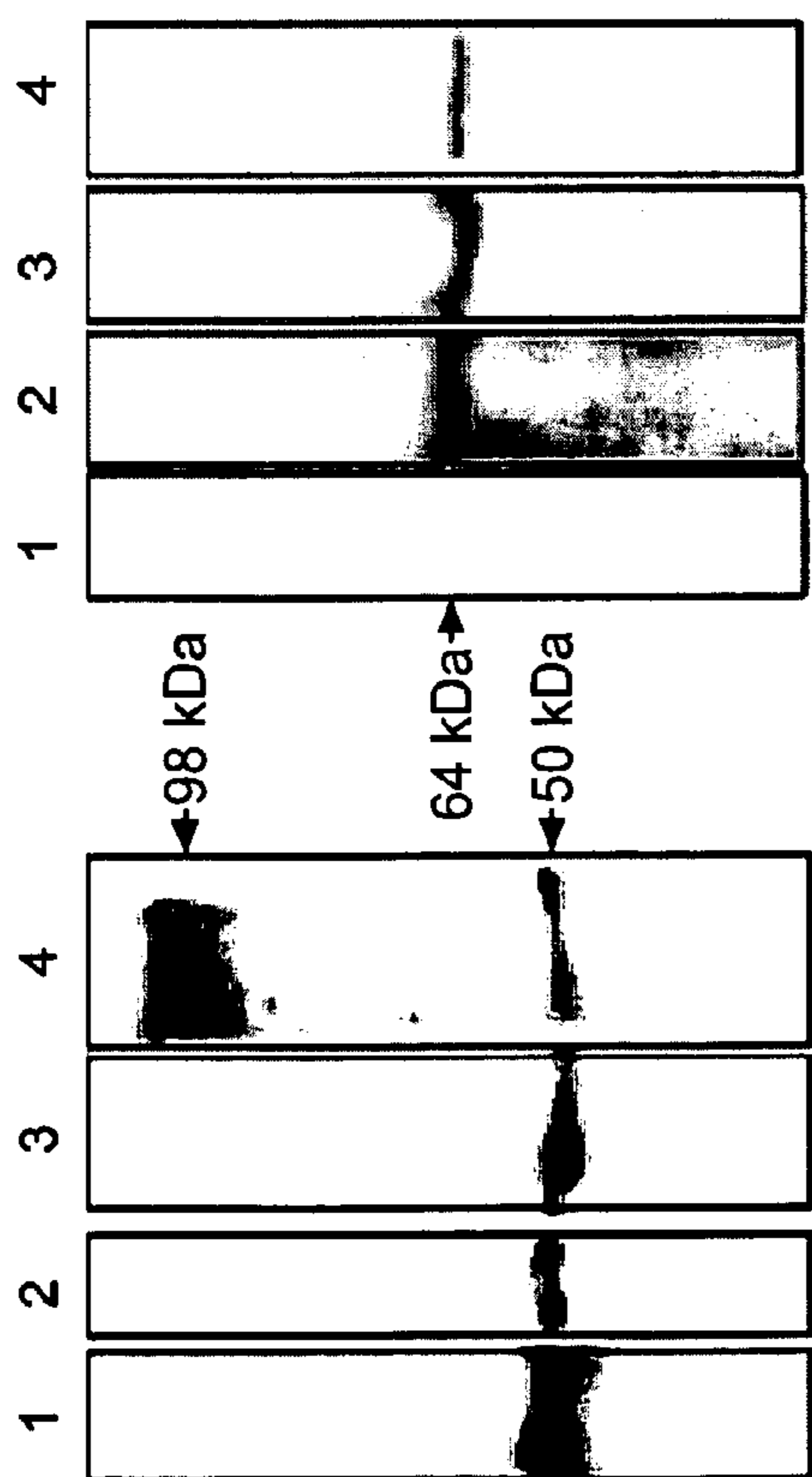


FIG. 3A

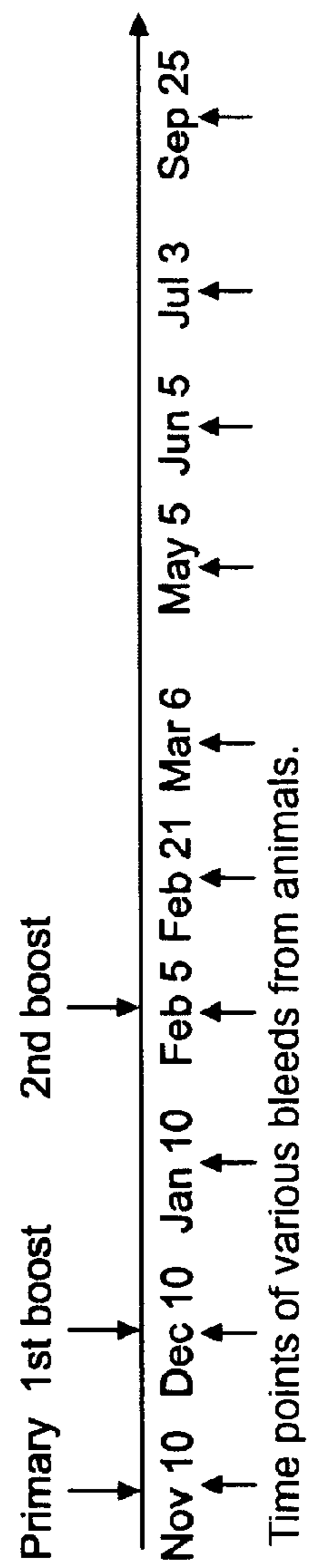


FIG. 4A

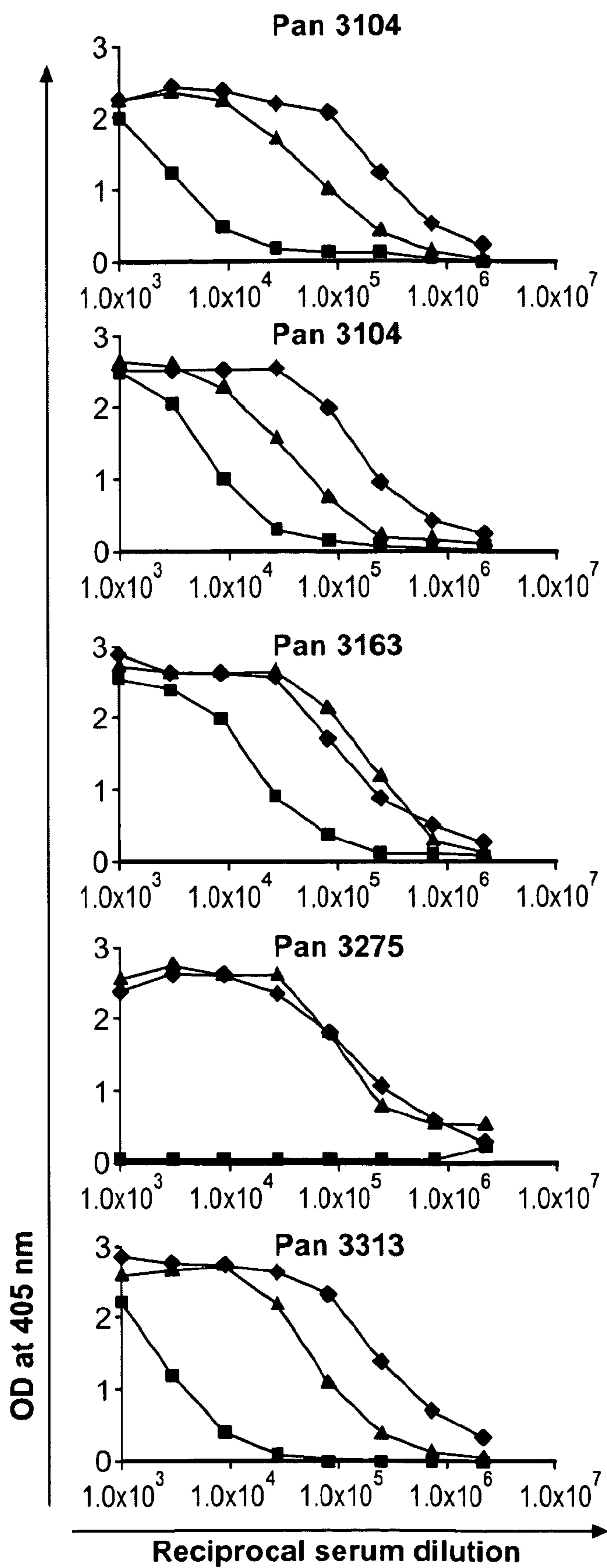


FIG. 4B

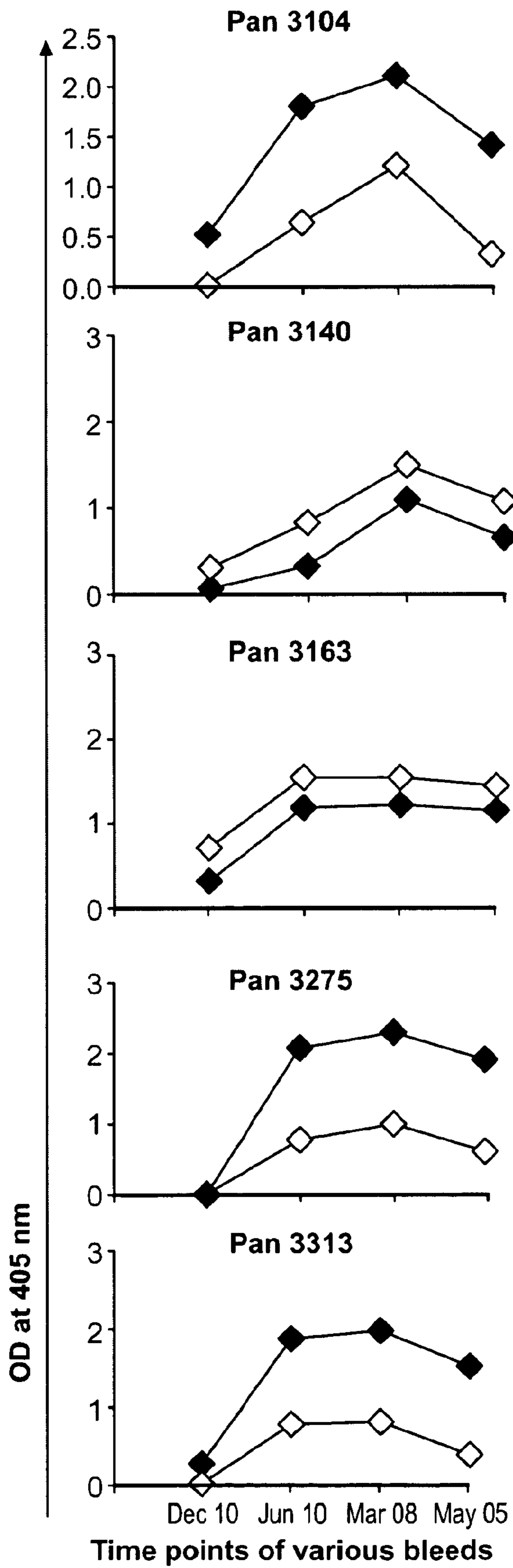
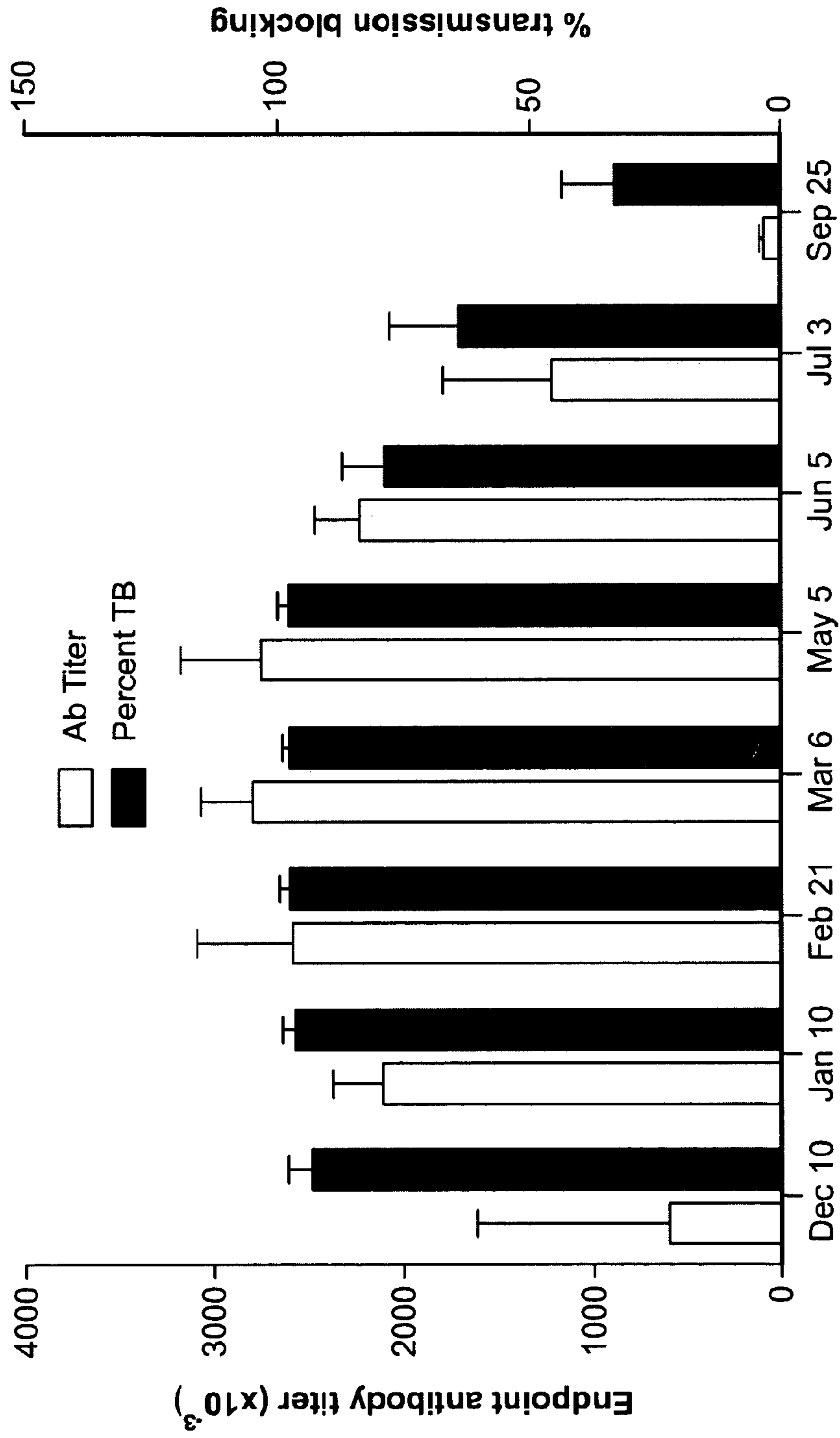


FIG. 4C



Various bleeds

FIG. 5

MALARIA VACCINE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 35 U.S.C. §371 U.S. national entry of International Application PCT/US2009/004243 (WO 2010/036293) having an International filing date of Jul. 22, 2009 which claims the benefit of U.S. Provisional Application No. 61/099,651, which was filed on Sep. 24, 2008, the entire contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Malaria is one of most dangerous infectious diseases in tropical and subtropical countries, afflicting about 300 million people. The pathogen of the disease is a protozoan parasite, *Plasmodium* sp. which is transmitted by *Anopheles* mosquitoes. Four species of malaria parasites can infect humans under natural conditions: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. *P. falciparum* and *P. vivax* cause the most infections worldwide.

P. falciparum is the agent of severe, potentially fatal malaria. Malaria caused by *P. falciparum* is responsible for nearly 1 million deaths annually. Based on recent estimates from the WHO, worldwide, there were an estimated 247 million malaria cases among 3.3 billion people at risk living in 109 countries [1]. Infections caused by *P. falciparum* and *P. vivax* account for more than 90% of global malaria burden; the former being responsible for nearly all the deaths due to malaria, nearly a million deaths of children under 5 years [2]. Among the current efforts against malaria include increasing use of insecticide treated bed nets and use of combination drugs to tackle the problem associated with drug resistance. The emergence of drug-resistant strains over the last 4 decades has underscored the necessity for improved control strategies. Accordingly, the development of a safe and effective malaria vaccine will be an important step towards controlling malaria. Vaccine development efforts to date have focused on candidate antigens represented in the pre-erythrocytic, erythrocytic and sexual stages of the parasite. Currently, the only vaccine advanced in clinical development, RTS,S, has shown partial protection against infection and disease severity in several clinical trials [6,7].

Immunity against the sexual stages of the parasite offers an effective way to reduce or stop malaria transmission and in that respect offers the greatest promise towards the goal of progressively eliminating malaria from endemic countries. A transmission blocking vaccine (TBV) [8] specifically targeting the sexual development of the parasite in the mosquito vector may elicit immunity which can effectively block transmission of the parasite from invertebrate mosquito vector to vertebrate host. Transmission of malaria depends upon the presence of infectious male and female gametocytes in the peripheral blood of infected persons and successful ingestion of these gametocytes by *Anopheles* mosquitoes. Soon after ingestion, exflagellation occurs within the mosquito midgut, and emergent male gametes fertilize female gametes, resulting in the formation of zygotes. The zygotes undergo post-fertilization transformation into motile ookinetes which traverse the midgut epithelium and develop into oocysts resulting in the production of infective sporozoites. Finally the sporozoites are released into the hemocoel, invade the salivary glands and are transmitted to vertebrate hosts during subsequent blood feeding [9].

The targets of transmission blocking antibodies include pre-fertilization antigens (Pfs230 and Pfs48/45) expressed in

the circulating gametocytes and post-fertilization antigens (Pfs25 and Pfs28) expressed during mosquito stage ookinete development [10]. Unlike Pfs25 and Pfs28, pre-fertilization antigens are also targets of the natural immune response and thus immunity induced by a vaccine based on any of these antigens will have the added benefit of natural boosting of immunity. To date, only Pfs25 and Pvs25 (*P. vivax* homolog of Pfs25) have undergone limited Phase I clinical trials with marginal success [11,12]. It has not been possible to evaluate any of the pre-fertilization antigens as vaccines simply because they have not been available in sufficient quantity and proper protein conformation.

Although much progress has been made in the recent past, the development of a safe, effective and affordable malaria vaccine has remained a challenge. A vaccine targeting sexual stages of the parasite will not only reduce malaria transmission by female *Anopheles* mosquitoes, but also reduce the spread of parasites able to evade immunity elicited by vaccines targeting pre-erythrocytic and erythrocytic asexual stages.

SUMMARY OF THE INVENTION

As described below, the present invention features immunogenic compositions based on pre-fertilization antigens expressed in the circulating gametocytes in the peripheral blood of infected persons. The present invention makes use of an approach that harmonizes codon usage frequency of the target gene with those of the expression host for heterologous expression of protein. Taking these concepts into account, an algorithm termed "codon harmonization" [19] was developed where synonymous codons from *E. coli* were selected that closely resemble the codon usage of a native pre-fertilization gene, for example the native Pfs48/45 gene, including regions coding 'link/end' segments of proteins in *P. falciparum*.

Also featured in the invention are antibodies, and methods to prevent the transmission of malaria using the immunogenic compositions of the invention.

In a first aspect, the invention features a method of blocking transmission of *Plasmodium falciparum* or *Plasmodium vivax* in a subject comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization antigens, thereby blocking transmission of *Plasmodium falciparum* or *Plasmodium vivax* in the subject.

In another aspect, the invention features a method of immunizing a subject against *Plasmodium falciparum* or *Plasmodium vivax* comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization antigens, thereby immunizing the subject against *Plasmodium falciparum* or *Plasmodium vivax*.

In still another aspect, the invention features a method for treating or preventing malaria in a subject comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization antigens, thereby preventing malaria in the subject.

In another aspect, the invention features a method of blocking transmission of *Plasmodium falciparum* or *Plasmodium vivax* in a subject comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* post-fertilization antigens, thereby blocking transmission of *Plasmodium falciparum* or *Plasmodium vivax* in the subject.

In one aspect, the invention features a method of immunizing a subject against *Plasmodium falciparum* or *Plasmodium*

vivax comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* post-fertilization antigens, thereby immunizing the subject against *Plasmodium falciparum* or *Plasmodium vivax*.

In another aspect, the invention features a method for treating or preventing malaria in a subject comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* post-fertilization antigens, thereby preventing malaria in the subject.

In one embodiment of any one of the above aspects, the one or more pre-fertilization or post-fertilization antigens are selected from the group consisting of Pfs48/45, Pfs 230, Pfs25, Pvs48/45, Pvs 230 and Pvs25.

In a further embodiment, the pre-fertilization antigen is Pfs48/45.

In another further embodiment, the post-fertilization antigen is Pfs25.

In another embodiment of any one of the above aspects, the one or more pre-fertilization antigens or post-fertilization antigens is derived from a codon harmonized gene. In a particular embodiment, the codon harmonized gene is encoded by the amino acid sequence corresponding to SEQ ID NO: 1. In another particular embodiment, the codon harmonized gene is encoded by the amino acid sequence corresponding to SEQ ID NO: 3. In still another particular embodiment, the codon harmonized gene is encoded by the amino acid sequence corresponding to SEQ ID NO: 5.

In another aspect, the present invention features a method of blocking transmission of *Plasmodium falciparum* or *Plasmodium vivax* in a subject comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* surface antigens, thereby blocking transmission of *Plasmodium falciparum* or *Plasmodium vivax* in the subject.

In another preferred aspect, the invention features a method of immunizing a subject against *Plasmodium falciparum* or *Plasmodium vivax* comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* surface antigens, thereby immunizing the subject against *Plasmodium falciparum* or *Plasmodium vivax*.

In still another aspect, the invention features a method for treating or preventing malaria in a subject comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* surface antigens, thereby preventing malaria in the subject.

In one embodiment of any one of the above aspects, the surface antigens are gametocyte or gamete surface antigens.

In a related embodiment, the gametocyte or gamete surface antigens are selected from the group consisting of: Pfs48/45, Pfs230, Pvs48/45 and Pvs230.

In another further embodiment of any one of the above aspects, the surface antigens are midgut parasite surface antigens. In a related embodiment, the midgut parasite surface antigens are selected from the group consisting of: Pfs25, Pfs28, Pvs25 and Pvs28.

In still another further embodiment of any one of the above aspects, the one or more surface antigens is derived from a codon harmonized gene.

In another related embodiment, the codon harmonized gene is encoded by the amino acid sequence corresponding to SEQ ID NO: 1. In another embodiment, the codon harmonized gene is encoded by the amino acid sequence corresponding to SEQ ID NO: 3. In another further embodiment,

the codon harmonized gene is encoded by the amino acid sequence corresponding to SEQ ID NO: 5.

In another further embodiment of any one of the above aspects, blocking transmission is measured by the reduction of mosquito oocysts by sera or plasma from a subject treated with the immunogenic composition compared to a control subject. In a further related embodiment, pre-immune sera from the treated subject and the control subject are used as a measure of 100% transmission for the corresponding test sera.

In another further embodiment of any one of the above aspects, the method further comprises administering an adjuvant. In a related embodiment, the adjuvant is selected from water-in-oil emulsion or Aluminum hydroxide.

In another further embodiment of any one of the above aspects, the method further comprises administering the immunogenic composition in one or more booster administrations. In a particular embodiment, the booster immunization is administered at 4 weeks. In another particular embodiment, the booster immunization is administered at 12 weeks.

In another further embodiment of any one of the above aspects, the method further comprises treatment with an additional agent. In a further embodiment, the additional agent is used to treat or prevent malaria.

In another further embodiment of any one of the above aspects, the composition is administered by one or more routes selected from the group consisting of: subcutaneous, intradermal, intramuscular, intravenous and transdermal delivery.

In still another further embodiment of any one of the above aspects, the composition is administered in a concentration between 1-100 µg.

In another aspect, the invention features an immunogenic composition comprising one or more pre-fertilization or post-fertilization antigens from *P. falciparum* or *P. vivax*.

In one embodiment, the one or more pre-fertilization or post-fertilization antigens are selected from the group consisting of Pfs48/45, Pfs230, Pfs 28, Pfs25, Pvs48/45, Pvs 230, Pvs 28, and Pvs25.

In a particular embodiment, the pre-fertilization antigen is Pfs48/45

In another particular embodiment, the post-fertilization antigen is Pfs25.

In still another further embodiment, the one or more pre-fertilization or post-fertilization antigens is derived from a codon harmonized gene.

In a further related embodiment, the codon harmonized gene encodes a protein represented by the amino acid sequence corresponding to SEQ ID NO: 1. In another embodiment, the codon harmonized gene is encoded by the amino acid sequence corresponding to SEQ ID NO: 3. In another further embodiment, the codon harmonized gene is encoded by the amino acid sequence corresponding to SEQ ID NO: 5.

In another further embodiment of any one of the above aspects, the composition is designed for expression in *E. coli*.

In another aspect, the invention features a vector comprising a codon harmonized Pfs48/45 sequence suitable for expression in a cell.

In still another aspect, the invention features a vector comprising a codon harmonized Pvs48/45 sequence suitable for expression in a cell.

In yet another aspect, the invention features a vector comprising a codon harmonized Pfs25 sequence suitable for expression in a cell.

In one embodiment, the codon harmonized Pfs48/45 sequence corresponds to the nucleic acid sequence compris-

ing SEQ ID NO: 2. In another embodiment, the codon harmonized Pfs25 sequence corresponds to the nucleic acid sequence comprising SEQ ID NO: 4. In still another embodiment, the codon harmonized Pvs48/45 sequence corresponds to the nucleic acid sequence comprising SEQ ID NO: 6.

In one embodiment of any one of the above aspects, the invention features a cell expressing the vector of any one of the aspects described.

In one embodiment, the cell is an *E. coli* cell or an *E. coli* derivative cell.

In another aspect, the invention features a kit comprising an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens, and instructions for use in reducing transmission of *Plasmodium falciparum* or *Plasmodium vivax*.

In yet another aspect, the invention features a kit comprising an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens, and instructions for use in preventing malaria.

In one embodiment, the one or more pre-fertilization or post-fertilization antigens are selected from the group consisting of Pfs48/45, Pfs230, Pfs 28, Pfs25, Pvs48/45, Pvs 230, Pvs 28, and Pvs25.

In another particular embodiment, the one or more pre-fertilization or post-fertilization antigens is derived from a codon harmonized gene.

In another aspect, the invention features a method for preparing a codon harmonized pre-fertilization or post-fertilization antigen sequence encoded by a *P. falciparum* or *P. vivax* pre-fertilization or post-fertilization gene comprising determining the frequency of codon usage of the pre-fertilization or post-fertilization gene coding sequence; and substituting codons in the coding sequence with codons of similar frequency from a host cell which code for the same pre-fertilization or post-fertilization antigen, thereby preparing a codon harmonized pre-fertilization or post-fertilization antigen sequence.

In one embodiment, the one or more pre-fertilization or post-fertilization genes are selected from the group consisting of Pfs48/45, Pfs230, Pfs 28, Pfs25, Pvs48/45, Pvs 230, Pvs 28, and Pvs25.

In another aspect, the invention features a method for preparing a codon harmonized Pfs48/45 antigen sequence encoded by a pre-fertilization or post-fertilization gene comprising determining the frequency of codon usage of the pre-fertilization or post-fertilization gene coding sequence, wherein the Pfs48/45 sequence corresponding to the nucleic acid sequence represented by SEQ ID NO: 7, and substituting codons in the coding sequence of SEQ ID NO: 7 with codons of similar frequency from a host cell which code for the Pfs48/45 antigen, thereby preparing a codon harmonized Pfs48/45 antigen sequence.

In one embodiment, the codon harmonized gene is expressed in a host cell.

In another embodiment, the host cell is *E. coli* or an *E. coli* derivative.

In still another embodiment, the invention features a codon harmonized nucleotide sequence prepared according to the methods described herein.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The following Detailed Description, given by way of example, but not intended to limit the invention to specific

embodiments described, may be understood in conjunction with the accompanying drawings, incorporated herein by reference.

Various preferred features and embodiments of the present invention will now be described by way of non-limiting example and with reference to the accompanying drawings in which:

FIG. 1 (a-f) shows the purification and characterization of CH-rPfs48/45. (a) Schematic representation of amino acid residues in the recombinant protein expressed. First 16 amino residues at the N-terminus contain 6× Histidine residues and a short linker to allow affinity purification of the protein. The sequence of Pfs48/45 begins with asparagine (N) at the 28 position and ends with serine (S) at 427 position in the native Pfs48/45 sequence. (b) Induction profile of CH-rPfs48/45. The cells were induced with 0.1 mM IPTG for 3 h and lysed by sonication. Lysates of uninduced and induced cells either non-reduced (left panel), or reduced (treated with 10 mM 2-mercaptoethanol, right panel) were run on SDS-polyacrylamide gel and stained with Coomassie stain. Lane 1; Protein molecular weight marker; lane 2, uninduced cell lysate; lane 3, induced cell lysate. The induced protein band in both non-reduced and reduced gels are encircled for easy recognition. (c) Flow diagram of various major steps including differential detergent extractions used for protein purification. (d) Western blot analysis for the presence of CH-rPfs48/45 at each step of detergent extraction using anti-His mAb. Lane 1, lysate pellet; lane 2, lysate supernatant; lane 3, 1% Tween-80 pellet; lane 4, 1% Tween-80 supernatant; lane 5, 0.5% sarcosyl pellet; lane 6, 0.5% sarcosyl supernatant. (e) Western blot analysis of purified CH-rPfs48/45 using anti-His mAb. Lane 1, eluate from Ni-NTA column; lane 2, dialyzed CH-rPfs48/45. (f) Recognition of CH-rPfs48/45 by conformation specific mAb IIC5B10. Lane 1, non-reduced CH-rPfs48/45; lane 2, reduced CH-rPfs48/45.

FIG. 2 (a & b) is two panels that show (a) ELISA analysis of CH-rPfs48/45 immunized individual mouse sera in three different adjuvant formulations: Complete Freund's adjuvant (top panel), Montanide ISA-51 (middle panel), and Alum (bottom panel). All the results are representative of three independent experiments. Pooled pre-immune sera+3SD are shown by broken lines. ELISA OD405 values for individual mice are shown; mouse 1 (filled triangle), mouse 2 (open square), mouse 3 (filled square), mouse 4 (open circle), mouse 5 (filled circle). (b) Analysis of anti-Pfs48/45 IgG isotype distribution in individual mouse sera: IgG1 (filled columns), IgG2a (hatched columns), IgG2b (stippled columns), IgG3 (blank columns).

FIG. 3 (a & b) shows recognition of native Pfs48/45 in *P. falciparum* gametocyte extract. (a) Western blot analysis with non-reduced (left panel) or reduced (right panel) *P. falciparum* gametocyte extract against serum of individual mouse immunized with either CFA or ISA-51 or alum formulation. Stage V gametocyte extract was run either in non-reduced or reduced (10 mM 2-mercaptoethanol) form in SDS-PAGE and transferred to nitrocellulose membrane. Mice sera were allowed to react at 1:1000 dilution for 1 h at 22° C. HRP-conjugated anti-mouse IgG at 1:10000 dilution was used as detection antibody and was developed using ECL substrate. Lane 1, mAb IIC5B10; lane 2, one representative mouse serum immunized in CFA; lane 3, one representative mouse serum immunized in Montanide ISA-51; lane 4, one representative mouse serum immunized in alum formulation. The figure is assembled from separate experiments. (b) Mouse sera (1:1000 dilution) were tested by live immunofluorescence assays as described under materials and methods.

FIG. 4 (a-c) shows analysis of anti-Pfs48/45 antibody production by Olive baboons (*Papio anubis*). (a) Each animal was immunized with CH-rPfs48/45 (50 μ g in 0.25 ml endotoxin free PBS) formulated in Montanide ISA-51 (0.25 ml) in baboons, administered intra muscularly (quadriceps, two sites). Schedules for immunization and bleeds are indicated and sera were stored at -20° C. until shipped frozen from Kenya to Baltimore for ELISA and MFA. The samples were shipped under an export permit CITES #008101. (b) Anti-Pfs48/45 whole IgG titer at various time points analyzed by ELISA. Pre-immune+3 \times SD is shown by solid horizontal lines. ELISA readings with sera dilutions, 1 month post primary immunization (filled square), 1 month post first boost (filled triangle) and 1 month post second boost (filled diamond) are shown with \pm SD for individual baboons (Pan 3104, Pan 3140, Pan 3163, Pan 3275, Pan 3313). (c) Distribution of anti-Pfs48/45 IgG1 (solid diamonds) and IgG2 (open diamonds) subtypes in 1 month post primary immunization sera (December 10), 1 month post 1st boost (January 10), 1 month post 2nd boost (March 06), and 3 months post 2nd boost (May 5). Data are presented as mean OD405 value \pm SD for individual baboon.

FIG. 5 is a graph that shows follow up of immune responses elicited by CH-rPfs48/45 in baboons. Analysis of anti-CH-rPfs48/45 IgG titers (open bars) and percent transmission blocking activity (closed bars) up to 7 months post second boost in baboons. Results show mean antibody titer and mean transmission blocking activity of 5 baboons+95% CI.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, for example, hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine, phosphothreonine.

The term "codon harmonization" is meant to refer to a process that harmonizes codon usage frequency of a target gene with those of the expression host for heterologous expression of protein. In preferred embodiments, codon harmonization refers to an algorithm where synonymous codons from *E. coli* are selected that closely resemble the codon usage of a native pre-implantation gene, for example the Pfs48/45 gene, including regions coding 'link/end' segments of proteins in *P. falciparum*.

By "fragment" is meant a portion (e.g., at least 10, 25, 50, 100, 125, 150, 200, 250, 300, 350, 400, or 500 amino acids or nucleic acids) of a protein or nucleic acid molecule that is substantially identical to a reference protein or nucleic acid and retains the biological activity of the reference. In some

embodiments the portion retains at least 50%, 75%, or 80%, or more preferably 90%, 95%, or even 99% of the biological activity of the reference protein or nucleic acid described herein.

The term "host cell" is meant to refer to a cell into which a foreign gene is introduced. The host cell can be prokaryotic or eukaryotic. In preferred embodiments, the host cell is *E. coli* or an *E. coli* derivative.

By "immunogenic composition" is meant to refer to one or more *Plasmodium* pre-fertilization or post-fertilization antigens that is capable of eliciting protection against malaria, whether partial or complete. An immunogenic composition may also be useful for treatment of an infected individual.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. Various levels of purity may be applied as needed according to this invention in the different methodologies set forth herein; the customary purity standards known in the art may be used if no standard is otherwise specified.

By "isolated nucleic acid molecule" is meant a nucleic acid (e.g., a DNA, RNA, or analog thereof) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule which is transcribed from a DNA molecule, as well as a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "nucleic acid" is meant an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid, or analog thereof. This term includes oligomers consisting of naturally occurring bases, sugars, and intersugar (backbone) linkages as well as oligomers having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of properties such as, for example, enhanced stability in the presence of nucleases.

The term "complimentary nucleic acid sequences" refer to contiguous DNA or RNA sequences which have compatible nucleotides (e.g., A/T, G/C) in corresponding positions, such that base pairing between the sequences occurs. For example, the sense and anti-sense strands of a double-stranded DNA helix are known in the art to be complimentary.

By "pre- or post-fertilization antigen" is meant to refer to a protein target expressed in *Plasmodium* that is necessary for *Plasmodium* transmission in a host. In particular embodiments, prefertilization antigens refer to antigens expressed in the intraerythrocytic gametocytes and male and female gametes prior to fertilization process. Examples include, but are not limited to, proteins such as Pfs230 and Pfs48/45 in *P. falciparum* or Pvs230 and Pvs48/45 in *P. vivax*. In other particular embodiments, post fertilization antigens refer to antigens expressed on the surface of zygotes formed after fertilization between female and male gametes and ookinetes. Examples include, but are not limited to, proteins such as Pfs25 and Pfs28 in *P. falciparum* and Pvs25 and Pvs28 in *P. vivax*.

By "protein" is meant any chain of amino acids, or analogs thereof, regardless of length or post-translational modification.

By “reference” is meant a standard or control condition.

By “specifically binds” is meant a molecule (e.g., peptide, polynucleotide) that recognizes and binds a protein or nucleic acid molecule of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a protein of the invention.

As used herein, the terms “treat,” “treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith, for example malaria. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

As used herein, the terms “prevent,” “preventing,” “prevention,” “prophylactic treatment” and the like refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition, for example malaria.

Other definitions appear in context throughout the disclosure.

Compositions

The present invention features immunogenic compositions comprising one or more pre-fertilization or post-fertilization antigens from *Plasmodium*, preferably *P. falciparum* or *P. vivax*. The genome of *Plasmodium falciparum* has been completely sequenced. (Gardner et al., Nature, 419:498-511 (2002)).

Preferably, the one or more of pre-fertilization or post-fertilization antigens are selected from the group consisting of Pfs48/45, Pfs230, Pfs 28, Pfs25, Pvs48/45, Pvs 230 Pvs 28, and Pvs25. Most preferably, the antigen is Pfs 48/45 or Pfs25 of *P. falciparum*. Alternatively, the antigen is Pvs 48/45 or Pvs25 of *P. vivax*.

Pfs230 and Pfs48/45 can be classified as gametocyte and gamete (male and female) surface antigens. Pfs25 and Pfs28 can be classified as mosquito midgut parasite stages (zygote and ookinete) surface antigens.

Targeted gene disruption studies have shown that Pfs48/45 plays a critical role in male gamete fertility, an important aspect of the sexual reproduction success of the parasite [14]. Analysis of immune human sera in endemic areas has also suggested a strong correlation between naturally present anti-Pfs48/45 antibodies and transmission reducing activity of those human sera; thus making it a key candidate for vaccine development [15]. However, efforts to produce full length recombinant Pfs48/45 in a functional conformation have largely remained unsuccessful. In a recent study, an approach that involved co-expression of a truncated version of C-terminal fragment of Pfs48/45 fused with a large fusion partner maltose binding protein (MBP) along with four periplasmic folding catalysts DsbA, DsbC, FkpA and SurA resulted in correctly folded truncated product which produced high titers of transmission-reducing antibodies in immunized BALB/c mice [16]. In this recombinant expression approach periplasmic targeting and folding into functional conformation of expressed Pfs48/45 protein was strictly dependent upon fusion with MBP as a carrier protein and protein folding was catalyzed by four chaperons co-expressed in the host, respectively.

Pfs25 is a *P. falciparum* antigen expressed on the surface of the malaria parasite. Pfs25 consists of four epidermal growth factor (EGF)-like domains located between a secretory signal sequence at the N-terminus, and a short C-terminal hydrophobic domain, which seems involved in the transfer of the EGF-like domains to a glycosyl-phosphatidylinositol (GPI) lipid anchor. There are 22 cysteine residues present as disulfide bonds in the four EGF-like domains of Pfs25. (Kaslow, et

al., Trends in Biotechnology, 10:388-391 (1992); Kaslow, et al., Nature, 333:74-76 (1988)). The disulfide bonds between the cysteine residues are essential for maintaining the structural integrity of the antigen. In an ex vivo experiment, antibodies to the antigen can completely block transmission of *P. falciparum*. (Vermeulen, et al., J. Exp. Med. 162:1460-1476 (1985)) The counterpart of Pfs25 in *P. vivax* is Pvs25.

It is a novel feature of the present invention that the one or more pre-fertilization or post-fertilization antigens is derived from a codon harmonized gene.

Codon Harmonization

It has been discovered that a nucleotide sequence capable of enhanced expression in host cells can be obtained by harmonizing the frequency of codon usage in the foreign gene at each codon in the coding sequence to that used by the host cell.

In certain embodiments, the invention features a nucleic acid sequence encoding a polypeptide to enhance expression and accumulation of the polypeptide in the host cell. Accordingly, the present invention provides novel nucleic acid sequences, encoding a polypeptide or protein that is foreign to a host cell, and that is expressed at greater levels and with greater biological activity than in the host cell as compared to the wild-type sequence if expressed in the same host cell.

Certain examples of codon harmonization have been described, for example, in US Application Nos. 20060088547 and 20080076161 and Angov et al. (PLOS, 2008. Volume 3, issue 5), which are incorporated by reference in their entireties herein.

The methods of the present invention, while directed to codon harmonization of pre- or post-fertilization antigens, are not limited as such, and are applicable to any coding sequence encoding a protein foreign to a host cell in which the protein is expressed.

Accordingly, in certain embodiments the invention features a method for preparing a codon harmonized pre-fertilization or post-fertilization antigen sequence encoded by *P. falciparum* or *P. vivax* a pre-fertilization or post-fertilization gene comprising determining the frequency of codon usage of the pre-fertilization or post-fertilization gene coding sequence, and substituting codons in the coding sequence with codons of similar frequency from a host cell which code for the same pre-fertilization or post-fertilization antigen, thereby preparing a codon harmonized pre-fertilization or post-fertilization antigen sequence.

For example, the frequency of occurrence of each codon in the *P. falciparum* or *P. vivax* pre-fertilization or post-fertilization gene of interest can be calculated and replaced with an *E. coli* codon with a similar frequency for the same amino acid. Preferably, the one or more pre-fertilization or post-fertilization genes are selected from the group consisting of Pfs48/45, Pfs230, Pfs 28, Pfs25, Pvs48/45, Pvs 230, Pvs 28, and Pvs25.

An existing DNA sequence can be used as the starting material and modified by standard mutagenesis methods that are known to those skilled in the art or a synthetic DNA sequence having the desired codons can be produced by known oligonucleotide synthesis, PCR amplification, and DNA ligation methods.

For example, the invention features a method for preparing a codon harmonized Pfs48/45 antigen sequence encoded by a pre-fertilization or post-fertilization gene comprising determining the frequency of codon usage of the pre-fertilization or post-fertilization gene coding sequence, wherein the Pfs48/45 sequence corresponding to the nucleic acid sequence represented by SEQ ID NO: 7, and substituting codons in the coding sequence of SEQ ID NO: 7 with codons

11

of similar frequency from a host cell which code for the Pfs48/45 antigen, thereby preparing a codon harmonized Pfs48/45 antigen sequence.

The native sequence of Pfs48/45 is represented by NCBI accession number AF356146, corresponding to SEQ ID NO: 7 shown below:

SEQ ID NO: 7

1 atgatgttat atatttctgc gaaaaaggct caagttgctt tcatcttata tatagtattg
61 gtattaagaa taataagtgg aaacaatgat ttttataatc ctagecgttt gaatagtgaa
121 atatctggat ttataggata taagtgtaat ttttcaaagtg aaggtgttca taatttaaag
181 ccagatatgc gtgaacgtag gtctatTTTT tgcaacatcc attcgtatTTT tatatatgat
241 aagataagat taataatacc taaaaaaagt tcgtcacctg agtttaaaat attacccgaa
301 aatgTTTTc aaaaagtata tactgattat gagaatagag ttgaaactga tatatcggaa
361 ttaggtTTTaa ttgaatatga aatagaagaa aatgatacaa accctaatta taatgaaagg
421 acaataacta tatctccatt tagtccaaaa gacattgaat ttttttgTTT ttgtgataat
481 actgaaaagg ttatatcaag tatagaaggg agaagtgcta tggtagatgt acgtgtatta
541 aaatatccac ataataTTTT atttactaat ttaacaaatg atctTTTTac atatttgccg
601 aaaacataata atgaatctaa ttttgtaagt aatgtattag aagtagaatt aaatgatgga
661 gaattatttg ttttagcttg tgaactaatt aataaaaaat gttttcaaga aggaaaagaa
721 aaagccttat ataaaagtaa taaaataatt tatcataata agttaactat ctttaaagct
781 ccattttatg ttacatcaaa agatgttaat acagaatgta catgcaaatt taaaaataat
841 aattataaaa tagtttttaa accaaaatat gaaaaaaaag tcatacacgg atgtaacttc
901 ttttcaaagtg ttagttctaa acatactttt acagatagtt tagatatttc tttagttgat
961 gatagtgcac atatttcatg taacgtacat ttgtctgaac caaaatataa tcatttggtg
1021 ggTTTTaaatt gtcttggtga tattatacca gattgctttt ttcaagtata tcaacctgaa
1081 tcagaagaac ttgaaccatc caacattggt tatttagatt cacaaataaa tataggagat
1141 attgaatatt atgaagatgc tgaaggagat gataaaatta aattatttgg tatagttgga
1201 agtataccaa aaacgacatc ttttacttgt atatgtaaga aggataaaaa aagtgcctat
1261 atgacagtta ctatagattc agcatattat ggatttttgg ctaaaacatt tatattccta
1321 attgtagcaa tattattata tatttag

DNA sequences modified by the method of the present invention are expressed at a greater level in host cells than the corresponding non-modified DNA sequence. Preferably, the host cell is *E. coli* or an *E. coli* derivative. The method can be applied to any DNA sequence for introduction into a host cell to provide protein product.

In particular exemplary embodiments of the present invention, the codon harmonized gene corresponds to the amino acid sequence corresponding to SEQ ID NO: 1, shown below, and the corresponding nucleic acid sequence, SEQ ID NO: 2.

SEQ ID NO: 1

NNDFYNPSALNSEISGFIGYKCNFSNEG VHNLPDMRERRSIFCNIIH
SYFIYDKIRLIIIPKSSSPEFKILPEKCFQKVYTDYENRVETDISEL
GLIEYEIEENDTNPYNERITITISPFSPKDIEFFCFDNTEKVISSI
EGRSAMVHVRVLKYPHNILFTNL TNDLFTYLPKTYNESNFVSNVLEV
ELNDGELFVLACELINKKCFQEGKEKALYKSNKIIYHNKLTIFKAPF

12

-continued

YVTSKDVNTECTCKFKNNNYKIVLKPKEYEKKVIHGCNFSNVSSKHT

5 FTDSLDISLVDDSAHISCNVHLSEPKYNHLVGLNCPGDIIPDCFFQV

-continued

YQPESELEPSNIVYLD SQINIGDIEYYEDAEGDDKIKLFGIVGSIP

50 KTTSTFTCICKDKKSAYMTVTIDS

SEQ ID NO: 2

AATAACGACTTCTACAACCCATCGGCTCTCAACTCTGAAATCAGCGG

55 CTTTCATCGGCTACAAGTGCAACTTCAGCAACGAAGGCGTTCACAACC

TGAAGCCAGACATGCGAGAACGACGAGCATTTTCTGTAATATACAC

TCGTACTTCATCTACGACAAGATCCGCTCTGATCATCCCAAAAAAAG

60 CTCGAGCCCAGAGTTCAAAATCCTGCCTGAAAAATGCTTCCAGAAAG

TTTACTACTGACTACGAGAACCCTGTTGAAACTGACATCTCGGAACTG

GGCCTGATTGAATACGAAATCGAAGAAAACGACACCAATCCAACTA

65 CAACGAACGCACCATCACGATCAGCCATTCTCTCCAAAAGATATTG

AATTCTTCTGCTTCTGCGACAACACTGAAAAGGTTATCAGCTCTATC

-continued

GAAGGGCGTTCTGCTATGGTTCACGTACGAGTTCTGAAATACCCACA
 CAACATTCTGTTCACTAACCTGACCAACGACCTCTTACCTACCTCC
 CTAAAACCTACAACGAAAGCAACTTCGTTTCTAACGTTCTGGAAGTT
 GAACTGAACGACGGCGAACTGTTGTTCTGGCTTGCGAACTCATTAA
 CAAAAATGCTTCCAGGAAGGCAAAGAAAAAGCCCTGTACAAATCTA
 AAAAAATCATTTACCACAACAAGCTCACTATATTCAAAGCTCCATTC
 TACGTTACCAGCAAAGACGTTAACACCGAATGCACCTGTAAATTCAA
 AAACAACAACCTACAAAATCGTTCTGAAACCAAATACGAAAAAAAAG
 TCATCCATGGCTGCAATTTTAGCAGCAACGTATCTAGCAAACACACT
 TTCACCGACTCTCTGGACATTAGCCTGGTTGACGACTCTGCTCACAT
 TAGCTGCAATGTTACCTCAGCGAACCAAATAACAACCACCTCGTTG
 GCCTGAACTGCCAGGCGACATTATCCAGACTGTTTCTTCCAGGTT
 TACCAGCCAGAAAGCGAAGAACTCGAACCATCGAATATTGTTTACCT
 GGACAGCCAGATCAACATCGGCGACATTGAATACTACGAAGACGCTG
 AAGGCGACGACAAAATTAACCTGTTGGCATCGTTGGCTCTATCCCA
 AAAACGACCAGCTTCACTTGCATCTGCAAGAAGGACAAAAAATCTGC
 TTACATGACCGTTACTATCGACTCT

SEQ ID NO: 2 preferably includes a signal and anchor sequence.

SEQ ID NO: 9 corresponds to the codon harmonized DNA sequence (SEQ ID NO:2, shown in italics) and restriction enzyme sites shown in bold, 6x histidine codons shown as underlined, as affinity tags, and linker sequences, preferably used to facilitate cloning.

SEQ ID NO: 9

CATATGGCACACCATCATCATCATCCCGGGGGATCCGGTTCTGG
 TACCAATAACGACTTCTACAACCCATCGGCTCTCAACTCTGAAATCA
 GCGGCTTCATCGGCTACAAGTGCAACTTCAGCAACGAAGGCGTTAC
 AACCTGAAGCCAGACATGCGAGAACGACGCAGCATTTTCTGTAATAT
 ACACTCGTACTTCATCTACGACAAGATCCGTCTGATCATCCCAAAAA
 AAAGCTCGAGCCCAGAGTTCAAAAATCCTGCCTGAAAAATGCTTCCAG
 AAAGTTTACTACTGACTACGAGAACCGTGTGAAACTGACATCTCGGA
 ACTGGGCTGATTGAATACGAAATCGAAGAAAACGACACCAATCCAA
 ACTACAACGAACGCACCATCACGATCAGCCATTCTCTCCAAAAGAT
 ATTGAATTCTTCTGCTTCTGCGACAACACTGAAAAGGTTATCAGCTC
 TATCGAAGGGCGTTCTGCTATGGTTCACGTACGAGTTCTGAAATACC
 CACACAACATTCTGTTCACTAACCTGACCAACGACCTCTTACCTAC
 CTCCCTAAAACCTACAACGAAAGCAACTTCGTTTCTAACGTTCTGGA
 AGTTGAACTGAACGACGGCGAACTGTTGTTCTGGCTTGCGAACTCA
 TTAACAAAAATGCTTCCAGGAAGGCAAAGAAAAAGCCCTGTACAAA
 TCTAACAAAAATCATTTACCACAACAAGCTCACTATATTCAAAGCTCC
 ATTCTACGTTACCAGCAAAGACGTTAACACCGAATGCACCTGTAAAT

-continued

TCAAAAACAACAACACTACAAAATCGTTCTGAAACCAAATAACGAAAAA
 AAAGTCATCCATGGCTGCAATTTTAGCAGCAACGTATCTAGCAAACA
 5 CACTTTCACCGACTCTCTGGACATTAGCCTGGTTGACGACTCTGCTC
 ACATTAGCTGCAATGTTACCTCAGCGAACCAAATAACAACCACCTC
 GTTGGCCTGAACTGCCAGGCGACATTATCCAGACTGTTTCTTCCA
 10 GGTTTACCAGCCAGAAAGCGAAGAACTCGAACCATCGAATATTGTTT
 ACCTGGACAGCCAGATCAACATCGGCGACATTGAATACTACGAAGAC
 GCTGAAGGCGACGACAAAATTAACCTGTTCCGGCATCGTTGGCTCTAT
 15 CCCAAAAACGACCAGCTTCACTTGCATCTGCAAGAAGGACAAAAAAT
 CTGCTTACATGACCGTTACTATCGACTCTTGATAAGCGGCCGC

In other embodiments of the invention, the codon harmonized gene corresponds to the amino acid sequence corresponding to SEQ ID NO: 3, shown below, and the corresponding nucleic acid sequence, SEQ ID NO: 4.

SEQ ID NO: 3

25 KYNNAKVTVDTVCKRGLIQMSGHLECKCENDLVINNEETCEEKVLKC
 DEKTVNKP CGDFSKCIKIDGNPVS YACKCNLGYDMVNNVCI PNECKNV
 TCGNGKCI LDTSNPVKTGVCSNIGKVPNVQDNKCSKDGETKCSLKC
 LKENETCKAVDGIYKCDCKDGFIDNESSICTAFSAYNILN

SEQ ID NO: 4

AAGTATAACAACGCCAAAGTTACTGTGACACTGTATGTAAACGTGGT
 TTCTTGATTCAAATGAGCGGCCACCTCGAATGCAAATGCGAAAACGAC
 35 CTGGTCTGGTAAACGAAGAAACCTGCGAAGAAAAAGTTTTAAATGC
 GATGAAAAGACTGTAAACAAACCGTGCAGTCTCTCGAAATGCATT
 AAAATTGACGGTAACCTGTTTCTTATGCTTGCAAATGCAACCTCGGT
 40 TACGACATGGTAAACAACGTTTGCATTCGAACGAATGCAAGAACGTA
 ACTTGCGGCAATGGCAAATGCATTCTGGACACCTCGAACCCAGTTAAA
 ACTGGTGTGTTGTTCTTGCAACATTGGGAAAGTTCTTAACGTACAGGAC
 45 CAGAACAAATGCTCTAAAGACGGTGAACGAAATGTTCTCTGAAATGT
 CTGAAAGAAAACGAAACGTGCAAAGCTGTTGACGGTATTTACAAATGC
 GACTGCAAAGACGGTTTCATTATTGACAACGAATCGAGCATTGCACT
 50 GCTTCTCTGCTTACAACATTCTGAAC

SEQ ID NO:4 preferably includes a signal and anchor sequence.

SEQ ID NO: 8 corresponds to the codon harmonized DNA sequence (SEQ ID NO:4, shown in italics) and restriction enzyme sites shown in bold, 6x histidine codons shown as underlined, as affinity tags, and linker sequences, preferably used to facilitate cloning.

SEQ ID NO: 8

CATATGGCACACCATCATCATCATCCCGGGGGATCCGGTTCTGGT
 ACCAAGTATAACAACGCCAAAGTTACTGTGACACTGTATGTAAACGT
 GGTTTCTGATTCAAATGAGCGGCCACCTCGAATGCAAATGCGAAAAC
 65 GACCTGGTCTGGTAAACGAAGAAACCTGCGAAGAAAAAGTTTTAAAA

-continued

TGCGATGAAAAGACTGTAAACAAACCGTGCGGTGACTTCTCGAAATGC
 ATTAAAATTGACGGTAACCCCTGTTTCTTATGCTTGCAAATGCAACCTC
 GGTACGACATGGTAAACAACGTTTGCATTCCGAACGAATGCAAGAAC
 GTAACCTGCGGCAATGGCAAATGCATTCTGGACACCTCGAACCCAGTT
 AAAACTGGTGTGTTGTTCTTGCAACATTGGGAAAGTTCCTAACGTACAG
 GACCAGAACAAATGCTCTAAAGACGGTGAAACGAAATGTTCTCTGAAA
 TGTCTGAAAGAAAACGAAACGTGCAAAGCTGTTGACGGTATTTACAAA
 TGCGACTGCAAAGACGGTTTCATTATTGACAACGAATCGAGCATTGTC
 ACTGCTTCTCTGCTTACAACATTCTGAACT**GATAAG**CGGCCGC

In other exemplary embodiments of the present invention, the codon harmonized gene corresponds to the amino acid sequence corresponding to SEQ ID NO: 5, shown below, and the corresponding nucleic acid sequence, SEQ ID NO: 6. SEQ ID NO: 5 shows the full length peptide sequence, where the signal peptide and the GPI anchor position are in bold.

MLKRQLANLLLVLVSLLRGITHTQMAKGEVKYVPEELNKDVS~~GF~~FK
 CNFSSKGVHNLEPILTEKRSLVCSIIYSYFIYDKIKLTI~~PK~~IPGSKFK
 MLPEKCFQTVYTYNIEKRTEEKIENMGLVEYEVKEDDSNSEYTEKILTI
 SPFNTKDVEFFCICDNSENVI~~SNV~~KGRVALVQVNV~~LKY~~PHKITSINLT
 KEPYSYLPNQVDKTSFKSHKLDLELQD~~ELV~~LACEKVD~~DKCF~~KKGKD
 TSPLSLYKSKKIVYHK~~NLS~~IFKAPVYVKSADVTAECSCNV~~DST~~IY~~TL~~S
 LKPVYTKKLIHG~~CNF~~SSDKSTHNFTNHV~~DMA~~ELGENAQITCSI~~EL~~VDT
 SYNHLIGM~~SCP~~GEVLPECF~~FQ~~VYQRESPELEPSKIVYL~~DAQ~~LNIG~~NVE~~
 YFEDSKGENIVKIFGLVGSIPK~~TS~~SFTCICR~~KG~~KKIGYMSV~~KIA~~AGYF
GFLAKIFILLIVLLLLLYF*

SEQ ID NO:6 corresponds to the codon harmonized Pvs48/45 DNA sequence consisting of 1274 bases, not including the signal sequence and minus GPI anchor sequence shown in SEQ ID NO:5.

SEQ ID NO: 6
CATATGGCACACCATCATCATCATCATCCCGGGGATCCGGTCTGGT
 ACCATGGCCAAGGGCGAGGTCAAATATGTGCCTCCAGAGGAGCTGAAT
 AAGGATGTGAGCGGCTTTTTTGGGTTAAGTGTAAATTTTAGCAGCAAG
 GGCGTCCATAACCTCGAGCCTATTCTGACGGAGAAGCGAAGCCTGGTC
 TGTAGCATTTATAGCTATTTTATTTATGATAAGATTA~~AA~~CTGACGATT
 CCTAAAAAATTCAGGCTCGAAGTTAAGATGCTCCAGAGAAGTGT
 TTTCAAACGGTGTATACTAATATGAGAAGCGCACGGAGGAGAAGATT
 GAGAATATGGGCCTCGTGGAGTATGAGGTGAAGGAGGATGATAGCAAC
 TCGGAGTATACGGAGAAGATTCTGACGATTAGCCCTTTTAAACGAAA
 GATGTGGAGTTTTTTTGTATTGTGATAACTCGGAGAACGTCATTAGC
 AATGTGAAAGGCCGAGTGGCGCTCGTGCAAGTGAATGTGCTCAAGTAT
 CCTCATAAGATTACGAGCATTAACTGACGAAAGAGCCATATTCGTAT

-continued

CTGCCTAATCAAGTGGATAAAAACGAGCTTTAAGTCGCATAAGCTCGAT
 CTCGAGCTCCAAGATGGCGAGCTCGTGGTGCTCGCGTGTGAGAAGGTG
 5 GATGATAAGTGTTTTAAAGAAAGGCAAAGATACTAGCCCCTGAGCCTC
 TATAAAAGCAAGAAGATTGTGTATCATAAAAACTCAGCATTTTTAAAG
 GCGCCTGTGTATGTGAAGAGCGCCGATGTGACGGCGGAGTGTAGCTGT
 10 AATGTGGATAGCACGATTTTATACTCTCAGCCTCAAGCCTGTGTATACG
 AAGAACTCATTCATGGGTGTAATTTTAGCTCGGATAAGAGCACGCAT
 AATTTTACTAATCATGTGGATATGGCCGAGCTGGGGGAGAATGCGCAA
 15 ATTACGTGTAGCATTGAGCTCGTGGATACGAGCTATAATCATCTCATT
 GGCATGAGCTGTCCTGGGGAGGTGCTGCCTGAGTGTTTTTTTCAAGTG
 TATCAACGAGAGAGCCCAGAGCTCGAGCCTAGCAAGATTGTCTATCTC
 GATGCCCAACTCAATATTGGCAATGTGGAGTATTTTGGAGTAGCAA
 20 GCGGAGAATATTGTGAAGATTTTGGGCTCGTGGGCAGCATTCCCTAAG
 ACGACGAGCTTTACGTGTATTTGTGAAAAGGGAAAAAGATTGGGTAT
 25 ATGAGCGTGAAGTGATAAGCGGCCGC

In still other embodiments of the present invention, the codon harmonized gene corresponds to the nucleic acid sequence shown as SEQ ID NO: 10, where SEQ ID NO: 10 is a codon harmonized Pfs230 C-terminal modified.

SEQ ID NO: 10
CATATGGCACACCATCATCATCATCATCCCGGGGATCCGGTCTGGT
 35 ACCAAAGAATATGTTTGCCTTCCAGCAGCTGAAACCGACCGAAT
 CTGGCCAAAAGTTAAAAATGCGAAGTTAAAGTTAACGAGCCGCTGA
 TCAAAGTTAAATCATCTGCCCGTGAAGGCAGCGTTGAAAACTGT
 40 ACGACAACATCGAATACGTTCCAAAAAATCGCCGTACGTTGTCTGA
 CCAAAGAGGAACTAACTCAAGGAAAACTGTTGTGCGAACTCATTT
 ACGGCCTGCTGATCAGCCCTACGGTTAATGAAAAGGAGAACAACCTCA
 45 AAGAAGCGTATTGAATCACTCTGCCTCCAGTGGTTCATAAGGCTA
 CCGTGTCTACTTTCATCTGCGACAACAGCAAAACCGAAGACGACAATA
 AAAAAGGCAACCGTGGGATTGTTGAAGTGTACGTTGAACCGTACGGCA
 50 ACAAATTAACGGCTGCGCTTTTCTCGACGAAGACGAAGAAGAAGAAA
 AATACGGCAACCAGATTGAAGAAGACGAACACACGAGAAGATCAAAA
 TGAAAACCTTTTTCACGCAAAACATCTACAAAAAACAACATCTACC
 CGTGCTACATGAAACTGTACTCGGGCGACATCGGCGGCAATCTCTTCC
 55 CAAAGAACATCAAAGCACCACGTGCTTGAAGAGATGATCCCATACA
 ACAAAGAAATCAAATGGAACAAAGAAAACAAATCTCTGGGCAATCTGG
 TTAACAACAGCGTTGTTTACAACAAGAGATGAACGCTAAATACTTCA
 60 ACGTTCAATACGTTTCATATTCCAACCTCTTACAAAGACACCCTGAACC
 TGTTCTGCTCTATTATCCTGAAAGAAGAGGAATCTAACCTGATTAGCA
 CTAGCTACCTGGTTTACGTTTCTATTAACGAAGAACTGAACTTCAGCC
 65 TCTTTGACTTCTACGAAAGCTTCGTTCCAATCAAAAAACGATCCAGG

-continued

TTGCTCAGAAGAACGTTAACAAACAAGAACACGACTACACCTGCGACT
 TCACGGACAAACTGGACAAAACGGTTCCAAGCAGTGTAAACGGGAAGA
 AACTGTTTATCTGCCGTAAGCACCTGAAAGAATTCGACACCTTACGCG
 TGAAATGCAACGTTAACAAAACCCAGTACCCGAACATAGAGATCTTCC
 CAAAAACCTGAAAGACAAAAGGAAGTTCTGAAACTGGACCTCGACA
 TCCAGTACCAGATGTTCTCTAAATTCTTCAAATTTAACACCCAAAACG
 CTAAGTACCTGAACCTGTACCCGTACTACCTGATTTTCCGTTCAACC
 ACATCGGCAAAAAAGAAGTGAACAAACCAACCTACAAAACCCACA
 AAGACGTGAAATACTTCGAGCAGAGCAGCGTTCTGAGCCCTCTGAGCT
 CGGCTGATTCTCTGGGAAACTGCTGAACTTCTGGACACTCAGGAGA
 CGGTTTGCCTCACGGAAAAGATCCGTTACCTGAACCTGTCTATAAACG
 AGCTGGGCAGCGACAACAACACCTTCAGCGTTACCTCCAAGTTCCGC
 CGTACATCGACATTAAGGAACCATTTACTTTCATGTTCCGGCTGCAACA
 ACAACAAAGGCGAAGGGAACATAGGCATTGTTGAACTGCTGATCAGCA
 AGCAGT**GATAAGCGGCCGC**

Here, the bold, underlined sequence refers to restriction enzyme sites to facilitate cloning into an expression plasmid vector.

In other embodiments of the present invention, the codon harmonized gene corresponds to the nucleic acid sequence shown as SEQ ID NO: 11, where SEQ ID NO: 11 corresponds to codon harmonized Pf230 A2B3.

SEQ ID NO: 11
CATATGGCACACCATCATCATCATCCCAGGGGATCCGGTTCTGGT
 ACCCAGACTACACCTGCGACTTACGGACAACTGGACAAAACGGTT
 CCAAGCACTGCTAACGGGAAGAACTGTTTATCTGCCGTAAGCACCTG
 AAAGAATTCGACACCTTACGCTGAAATGCAACGTTAACAAAACCCAG
 TACCCGAACATAGAGATCTTCCAAAAACCTGAAAGACAAAAGGAA
 GTTCTGAAACTGGACCTCGACATCCAGTACCAGATGTTCTCTAAATTC
 TTCAAATTTAACACCCAAAACGCTAAGTACCTGAACCTGTACCCGTAC
 TACCTGATTTTCCCGTTCAACACATCGGCAAAAAGAACTGAAAAAC
 AACCAACCTACAAAACCAAAAGACGTGAAATACTTCGAGCAGAGC
 AGCGTTCTGAGCCCTCTGAGCTCGGCTGATTCTCTGGGAAACTGCTG
 AACTTCCTGGACACTCAGGAGACGGTTTGCCTGACGAAAAGATCCGT
 TACCTGAACCTGTCTATAAACGAGCTGGGCAGCGACAACAACACCTTC
 AGCGTTACCTTCCAAGTCCGCGTACATCGACATTAAGGAACCATTC
 TACTTCATGTTCCGGCTGCAACAACAAGGCGAAGGGAACATAGGC
 ATTGTTGAACTGCTGATCAGCAAGCAGGAAGAAAAGATTAAGGCTGC
 AACTTTCACGAAAGCAAACCTGACTACTTTAACGAAAATATTAGCTCT
 GACACCCACGAATGCACCTTCACGCTTACGAAAACGACATCATTGGC
 TTCAACTGCCTGGAACTACTCACCCAAACGAGGTTGAGGTTGAAGTT
 GAAGACGCTGAAATCTACCTCCAGCCAGAGAAGTCTTCAACAACGTT
 TACAAAGGCCTCAACAGCGTTGACATTACTATCTGAAAAACGCT

-continued

CAGACCTACAACATCAACAACAAGAAAACCCCAACGTTCTGAAAATT
 CCGCCGTACAACCTGCTGGAAGACGTCGAAATTTCTTGTGTCAGTGCCT
 5 ATTAACAGGTTGTTAAAAAATCAAAGTTATTATCACGAAAAACGAC
 ACCGTTCTGCTGAAACGTGAAGTGCAGAGCGAGAGCACCCCTGGACGAC
 AAAATCTACAATGCGAACACGAAAACCTCATTAAACCCGCGTGTAAAC
 10 AAAACCTTCGACGAAAACGTTGAATACACCTGCAACATCAAAATCGAG
 AACTTTTTCAACTACATTGAGATCTTCTGCCCGGCCAAAGACCTCGGC
 ATTTACAAAACATCCAGATGTACTACGACATTGTTAAACCGACCCGT
 15 GTTCCGCGATTCAAAAAATTCAACAACGAAGAACTGCACAACTGATT
 CCAAACAGCGAAATGCTGCACAAAACCAAAGAAATGCTGATTCTGTAC
 AACGAAGAAAAGTGGACCTCCTGCACTTCTACGTTTTTCTGCCGATC
 20 TACATCAAAGATATCTACGAATTTAACATCGTTTTCGACAACAGCAAA
 ACCATGTGAAAAACAGCTGGGCGGCAAAGTTATCTACCACATTACT
 GTTAGCAAACGTGAGCAAAAAGTTAAAGGCTGCAGCTTCGACAACGAA
 25 CACGCTCACATGTTCTCTTACAACAAAACCTAACGTTAAAACTGCATT
 ATCGACGCTAAACCAAAGACCTCATCGGCTTTGTTTGCCCTAGCGGC
 ACGCTGAAACTGACCAACTGCTTCAAAGACGCTATCGTTACACCAAC
 30 CTGACCAACATTAACGGCATCCTCTACCTGAAAAACAACCTCGCTAAT
 TTCACCTACAAACACCAGTTCAACTACATGAAATCCCGGCTCTGATG
 GACAACGACATCAGCTTCAAATGCATCTGCGTTGACCTGAAAAA
 35 AAATACAACGTCAAAAGCCCGCTGGGCC**ATGATAAGCGGCCGC**

Here, the bold, underlined sequence refers to restriction enzyme sites to facilitate cloning into an expression plasmid vector.

In still other embodiments of the present invention, the codon harmonized gene corresponds to the nucleic acid sequence shown as SEQ ID NO: 12, where SEQ ID NO: 12 corresponds to codon harmonized Pfs230 A4B5.

SEQ ID NO: 12
CATATGGCACACCATCATCATCATCCCAGGGGATCCGGTTCTGGT
 ACCAACCGTACGTTTTCGACTTTAGCAAAAACAACCTGATTGTTCCG
 GAAAGCCTGAAAAAAAAGAAGAGCTCGGCGGCAACCCGGTTAACATT
 50 CACTGCTACGCTCTGCTGAAACCACTGGACACCCCTGTACGTTAAATGC
 CCAACCAGCAAAGACAACACTACGAAGCTGCTAAAGTTAATATCAGCGAA
 AATGATAACGAATACGAGCTGCAGGTTATCAGCTGATAGAAAAACGT
 55 TTCCACAACCTTCGAGACGCTGGAATCGAAGAAACAGGCAACGGCGAC
 GTTGTGTTTCAACCGGCGTTGTTGACACTGGCCAGTTCTGGACAAT
 TCTACCTTCGAAAAATACTTCAAAAACATCAAAATCAAACCGACAAA
 60 TTCTTCGAGAAAGTTATCAACGAATACGACGACACTGAAGAAGAAAA
 GACCTGGAATCTATCCTGCCAGGGGTATTGTTTCTCCAATGAAAGTT
 CTGAAAAAAGGACCCATTACCAGCTACGCTGCTTTCGTTGTTCCG
 65 CCGATTGTTCTAAAGACCTGCACTTCAAAGTTGAATGCAACAACACC

-continued

GAATACAAAGACGAAAACAGTACATCTCTGGCTACAACGGCATCATC
CACATTGACATCAGCAACTCTAACCGCAAATTAACGGCTGCGACTTT
AGCATAATAACTCTAGCATTCTGACCTCGTCTGTTAACTGGTTAAC
5 GCGGAACTAAAACTGCGAAATCAACATCAACAACAACGAAGTTTTC
GGCATAATCTGCGACAACGAAACCAACCTGGACCCGAAAAATGCTTC
CACGAAATCTACTCTAAAGACAACAAAATGTTAAAAAATCCGAGAA
10 GTTATCCAAACATCGACATCTTTAGCCTGCACAACGACAACAAGAAA
AAAGTTGCTTACGCTAAAGTTCCACTGGACTACATTAACAAAATGCTG
TTCAGCTGCAGCTGCAAAACAGCCACACTAACCCATCGGCACGATG
AAAGTTACTCTCAACAAGACGAAAAAGAAGAAGACTTCAAAACC
GCTCAGGGCATTAAACACAACAACGTTACCTGTGCAACTTTTTCGAC
AACCCAGAACTGACCTTCGACAACAACAATCGTTCTGTGCAAAATA
GACGCTGAATTATTTAGCGAAGTTATTATCCAGCTGCCGATCTTCGGC
ACCAAGAAGCTTGAAGAAGGCTTCAGAACGAAGAATACAAAAATTC
AGCCTGAAACCGAGCCTGGTTTCGACGACAATAACAACGACATTAAG
TTATCGGCAAAGAAAAAAGCAAGTTAGCATTCTCTGGCTCTCAAAG
GGGTTTACGGCAACCGAATTTTCACTTTTCGACAAAAACGGCAAAAAAG
GCGAAGGCATTTCTTCTCATCCACCGATCAACAGGACACCGACC
TGAAATTCATCATTAAACGAAACCATCGACAACAGCAACATTAACAGC
GTGGCCTGATCTACATTTTCGTTTCGAAAAACGTTAGCGAAAAACAGCT
TCAAACCTGTGCGACTTTACCACCGCTCGACTAGCCTGATGGAACCTGA
ACTCTCAGGTTAAAGAAAAAAGTGTACTGTTAAAAATAAAAAAGGCG
ACATTTTCGGCCTCAAATGCCAAAAGGCTTCGCTATCTTCCCGCAGG
CTTGCTTCTCTAACGTTCTGCTGGAATACTACAAATCTGACTACGAAG
ACTCTGAACACATTAACACTACTACATTCACAAAGACAAAAATACAACC
TGAAACCAAAGACGTTATTGAACTGATGGACGAAAACTTCCGTGAAC
TGCAGAACATCCAGCAGTACACCGGCATCAGCAACATTACCGACGTGC
TGCACTTTAAAAACTTCAACCTGGGCAACCTCCCGCTGAACTTCAAAA
ACCACTACAGCACCGCTTACGCTAAAGTTCCGGACACGTTCAACAGCA
TTATTAATTTTAGCTGCAACTGCTACAACCCGAAAAACACGTTTACG
GCACTATGCAGGTTGAGAGCGACAAC**TGATAAGCGGCCGC**

Here, the bold, underlined sequence refers to restriction enzyme sites to facilitate cloning into an expression plasmid vector.

In other embodiments of the present invention, the codon harmonized gene corresponds to the nucleic acid sequence shown as SEQ ID NO: 13, where SEQ ID NO: 13 corresponds to codon harmonized Pfs230 A6B7.

SEQ ID NO: 13

CATATGGCACACCATCATCATCATCCCGGGGGATCCGGTTCTGGT
ACCAACGAACACATCTGCGACTACGAAAAAACGAAAGCTTAATCAGC
ACCCTGCCAAACGACACCAAAAAAATCCAGAAATCTATATGCAAAATT
AACGCTAAAGCTCTGGACGTTGTTACCATTAAATGCCACACACCAA

-continued

AACTTCACGCCAAAAGACTACTTCCCAAACAGCAGCCTGATCACTAAC
GACAAAAAATTTGTGATTACTTTTCGACAAGAAAAACTTCGTTACTTAC
5 ATCGACCCAACCAAAAAACCTTCAGCCTCAAAGACATCTACATCCAG
TCTTTCTACGGCGTTAGCCTCGACCACCTCAACCAGATCAAAAAATC
CACGAAGAATGGGACGACGTTACCTGTTCTACCCACCACACAACGTT
10 CTGCACAACGTTGTTCTCAACAACCACATCGTCAATCTGAGCAGCGCT
CTGGAAGGCGTCTGTTTATGAAAAGCAAAGTTACTGGCGACGAAACC
GCTACCAAAAAAATACTACCCTCCCGACTGACGGCGTTAGCTCTATT
15 CTGATTCCGCCGTACGTTAAGGAAGACATCACCTTCCACCTCTTCTGC
GGGAAAAGCACCACCAAAAAACCGAATAAAAAAGAATACCAGCCTCGCT
CTCATTACATCCACATCAGCAGCAATCGTAACATTATTACGGCTGC
20 GACTTTCTGTACCTGGAAAACAGACCAACGACGCTATTTCTAACAAAC
AACAAACAGCTACAGCATCTTCAACCACAACAAAAACACCGAGAAC
AACCTCATCTGCGACATCAGCCTGATTCGAAAACTGTTATCGGCATT
25 AAATGCCCAAACAAAAAAGTGAACCCGACAGCCTGCTTCGACGAAGTG
TACTACGTTAAACAGGAAGACGTTCCATCGAAAACTATCACCGCTGAC
AAATAACAACCTTCTCTAAAGATAAAAAATCGGCAACATCTGAAAAAC
30 GCTATAAGCATTAAACAACCCGACGAAAAGGACAACACCTACACTTAC
CTGATCCTGCCGAAAAATTCGAAGAAGAACTGATAGACACGAAAAAA
GTTCTGGCTTGACCTGCGACAACAAATACATCATCCACATGAAAATC
35 GAAAAATCTACCATGGACAAAATCAAAATCGACGAAAAAAAACCATTT
GGCAAAGACATCTGCAAATACGACGTTACTACTAAAGTTGCTACTTGC
GAAATTATTGACACCATTGACTCGAGCGTTCTGAAAGAACCACACACC
40 GTTCACTACAGCATTACCCTGAGCCGTTGGGACAAACTCATTATTTAA
TACCCGACCAACGAGAAAACCCACTTTGAAAACTTCTTCGTTAACCCA
TTCAACCTGAAAGACAAAGTTCTGTACAACCTACAACAACCGATCAAC
45 ATCGAACACATACTGCCGGCGCCATTACCACCGACATCTACGACACG
CGTACCAAAATTAACAGTACATCCTGCGTATTCCGCCGTACGTTTAC
AAAGACATCCACTTTAGCCTGGAATTCAATAACTCGCTCTCTGACC
AAACAGAACCAGAACATTATTTACGGCAACGTTGCCAAAATTTTCATT
50 CACATCAACCAGGGCTACAAAGAAATTCACGGCTGCGACTTTACCGGC
AAATACTCGCACCTGTTTACCTACAGCAAAAAACCACTGCCGAACGAC
GACGACATCTGCAACGTTACTATCGGCAACAACACCTTTAGCGGCTTC
55 GCTTGTCTGTGCACTTCGAACTGAAACCGAACAATTGTTTTAGCAGC
GTTTACGACTACAACGAAGCCAACAAGTTAAAAAATGTTTACCTC
TCGACCAAAGTTGAACTGGATCACATAAAACAGAACACTAGCGGCTAC
60 ACCCTCAGCTACATTTTCAACAAGAAATCGACCAAACTCAAATTT
AGCTGCACCTGTAGCTCGAATTACAGCAACTACACTATCCGAATAACC
TTCGACCCAT**TGATAAGCGGCCGC**

Here, the bold, underlined sequence refers to restriction enzyme sites to facilitate cloning into an expression plasmid vector.

The compositions of the invention are designed for expression in a host. In preferred embodiments, a host is *E. coli* or an *E. coli* derivative. The DNA encoding the desired recombinant protein can be introduced into a host cell in any suitable form including, the fragment alone, a linearized plasmid, a circular plasmid, a plasmid capable of replication, an episome, RNA, etc. Preferably, the gene is contained in a plasmid. In a particularly preferred embodiment, the plasmid is an expression vector. Individual expression vectors capable of expressing the genetic material can be produced using standard recombinant techniques. Please see e.g., Maniatis et al., 1985 Molecular Cloning: A Laboratory Manual or DNA Cloning, Vol. I and II (D. N. Glover, ed., 1985) for general cloning methods

Accordingly, the present invention contemplates host cells transformed with a vector as described herein. For example, the vector may comprise a codon harmonized Pfs48/45 sequence suitable for expression in a cell, a codon harmonized Pvs48/45 sequence suitable for expression in a cell, a codon harmonized Pfs25 sequence suitable for expression in a cell.

The one or more *Plasmodium* pre-fertilization or post-fertilization antigens, for example *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens can be used singly, or in combinations. For example, a combination may comprise antigens derived from different *Plasmodium* species that will be capable of blocking the infection and/or transmission of more than one *Plasmodium* species. For example, an immunogenic composition comprising Pfs48/45 and Pvs48/45 will be capable of blocking the transmission of both *P. falciparum* and *P. vivax*.

METHODS OF THE INVENTION

The present invention describes an approach that harmonizes codon usage frequency of the target gene with those of the expression host for heterologous expression of protein. Basic studies on regulation of protein expression have shown that synonymous codon substitutions from infrequent to frequent usage in regions where mRNA translation occurs relatively slowly can be detrimental to protein expression and stability [17]. On the other hand, codon substitutions introducing rare codons in the regions containing high frequency codons can lead to erroneous protein conformation [18]. Taking these concepts into account, an algorithm termed "codon harmonization" [19] was developed where synonymous codons from *E. coli* were selected that closely resemble the codon usage of native Pfs48/45 gene, including regions coding 'link/end' segments of proteins in *P. falciparum*. This approach harmoniously mimics the translation rate of protein expression in native host by allowing the translation machinery to pause at exactly the same positions in *E. coli* as in *P. falciparum* and yield expression of correctly folded protein, for example, Pfs48/45 in *E. coli*.

The present invention describes for the first time the efficient and successful expression of a pre-fertilization antigen, and in particular, full length TBV candidate Pfs48/45, in high yields and appropriate conformation. The recombinant Pfs48/45 elicits potent malaria transmission blocking antibodies in mice and non human primates (Olive baboons, *Papio anubis*) and indicates the development of a malaria TBV based on the CH-rPfs48/45 antigen.

In certain preferred embodiments of the invention, the pre-fertilization or post-fertilization antigens can be used to develop malaria transmission-blocking immunogenic compositions. Accordingly, the invention features methods of blocking transmission of *Plasmodium falciparum* or *Plasmo-*

dium vivax in a subject comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens, thereby blocking transmission of *Plasmodium falciparum* or *Plasmodium vivax* in the subject.

In certain embodiments, it may be desirable to measure transmission, for example, by measuring the reduction of mosquito oocysts by sera or plasma from a subject treated with the immunogenic composition compared to a control subject. Accordingly, pre-immune sera from the treated subject and the control subject are used as a measure of 100% transmission for the corresponding test sera.

The invention also features methods of immunizing a subject against *Plasmodium falciparum* or *Plasmodium vivax* comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens, and thereby immunizing the subject against *Plasmodium falciparum* or *Plasmodium vivax*.

The immunogenic compositions of the invention are also preferably used in methods for treating or preventing malaria in a subject comprising administering to a subject an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens, thereby preventing malaria in the subject.

In preferred embodiments, the pre-fertilization or post-fertilization antigens can be classified as *Plasmodium falciparum* or *Plasmodium vivax* surface antigens. In certain preferred embodiments, the surface antigens are gametocyte or gamete surface antigens. Exemplary gametocyte or gamete surface antigens are selected from the group consisting of Pfs48/45, Pfs230, Pvs48/45 and Pvs230. In other embodiments, the surface antigens are midgut parasite surface antigens. Exemplary midgut parasite surface antigens are selected from the group consisting of Pfs25, Pfs28, Pvs25 and Pvs28.

Preferably, the one or more pre-fertilization or post-fertilization antigens are selected from the group consisting of Pfs48/45, Pfs 230, Pfs25, Pvs48/45, Pvs 230 and Pvs25. In particular, in certain embodiments, the pre-fertilization antigen is Pfs48/45. In other embodiments, the post-fertilization antigen is Pfs25.

As described herein, codon harmonized genes are preferably employed in the methods of the invention, where, generally, a wild type nucleic acid sequence encoding a polypeptide is modified to enhance expression and accumulation of the polypeptide in the host cell by harmonizing synonymous codon usage frequency between the foreign DNA and the host cell DNA. Accordingly, in certain preferred embodiments, of the invention, one or more pre-fertilization antigens or post-fertilization antigens is derived from a codon harmonized gene.

Codon harmonized genes are described herein, and in particular embodiments the modified (codon harmonized) nucleic acid sequence and/or the encoded polypeptide are shown in SEQ ID NOs 1-SEQ ID NO: 13.

Administration

The immunogenic compositions of the present invention can be administered to a subject by different routes such as subcutaneous, intradermal, intramuscular, intravenous and transdermal delivery. Suitable dosing regimens are preferably determined taking into account factors well known in the art including age, weight, sex and medical condition of the subject; the route of administration; the desired effect; and the particular composition.

The course of the immunization may be followed by assays for activated T cells produced, skin-test reactivity, antibody formation or other indicators of an immune response to a malarial strain.

Dosage form, such as injectable preparations (solutions, suspensions, emulsions, solids to be dissolved when used, etc.), tablets, capsules, granules, powders, liquids, liposome inclusions, ointments, gels, external powders, sprays, inhalation powders, eye drops, eye ointments, and the like, can be used appropriately depending on the administration method. Pharmaceutical formulations are generally known in the art and are described, for example, in Chapter 25.2 of Comprehensive Medicinal Chemistry, Volume 5, Editor Hansch et al, Pergamon Press 1990.

Pharmaceutically acceptable carriers which can be used in the present invention include, but are not limited to, an excipient, a stabilizer, a binder, a lubricant, a colorant, a disintegrant, a buffer, an isotonic agent, a preservative, an anesthetic, and the like which are commonly used in a medical field.

Immunogenic compositions are administered in immunologically effective amounts. An immunologically effective amount is one that stimulates the immune system of the subject to establish a level of immunological response sufficient to reduce parasite density and disease burden caused by infection with the pathogen, and/or sufficient to block the transmission of the pathogen in a subject.

A dose of the immunogenic composition may, in certain preferred embodiments, consist of the range of 1 µg to 1.0 mg total protein. In certain preferred embodiments, the composition is administered in a concentration between 1-100 µg. However, one may prefer to adjust dosage based on the amount of antigen delivered. In either case these ranges are guidelines. More precise dosages should be determined by assessing the immunogenicity of the composition so that an immunologically effective dose is delivered. The immunogenic composition can be used in multi-dose formats.

The timing of doses depends upon factors well known in the art. After the initial administration one or more booster doses may subsequently be administered to maintain antibody titers, e.g., the compositions of the present invention can be administered one time or serially over the course of a period of days, weeks, months and or years. An example of a dosing regime would be day 1 an additional booster doses at distant times as needed. The booster doses may be administered at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more weeks after the primary immunization. In preferred embodiments, the booster doses are administered at 4 weeks. In other preferred embodiments, the booster doses are administered at 12 weeks.

In other cases, the immunogenic compositions are administered after, before or at the same time as treatment with an additional agent, such as an agent to treat or prevent malaria.

As used herein the subject that would benefit from the immunogenic compositions described herein include any host that can benefit from protection against malarial infection. Preferably, a subject can respond to inoculation with the immunogenic compositions of the present invention by generating an immune response. The immune response can be completely or partially protective against symptoms caused by infection with a pathogen such as *Plasmodium falciparum*, or can block transmission of the pathogen by *Anopheles* mosquitoes. In a preferred embodiment, the subject is a human. In another embodiment, the subject is a non-human primate.

Formulations

The immunogenic compositions of the present invention can be used to immunize mammals including humans against infection and/or transmission of malaria parasite, or to treat

humans post-infection, or to boost a pathogen-neutralizing immune response in a human afflicted with infection of malaria parasite.

The immunogenic compositions of the present invention can be formulated according to methods known and used in the art. Guidelines for pharmaceutical administration in general are provided in, for example, Modern Vaccinology, Ed. Kurstak, Plenum Med. Co. 1994; Remington's Pharmaceutical Sciences 18th Edition, Ed. Gennaro, Mack Publishing, 1990; and Modern Pharmaceutics 2nd Edition, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990. Immunogenic compositions of the present invention can be prepared as various salts. Pharmaceutically acceptable salts (in the form of water- or oil-soluble or dispersible products) include conventional non-toxic salts or the quaternary ammonium salts that are formed, e.g., from inorganic or organic acids or bases. Examples of such salts include acid addition salts such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate; and base salts such as ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as histidine, arginine and lysine.

Adjuvants are almost always required to enhance and/or properly direct the immune response to a given antigen. An ideal adjuvant should be safe, stable with long shelf life, biodegradable, inexpensive and promote an appropriate immune response while itself being immunologically inert. Adjuvants affect processes including antigen presentation, antigen uptake and selective targeting of antigens thus critically determining the magnitude and type of the immune responses [34-36]. While the mechanisms by which different adjuvants result in different outcomes remain a "black box", studies strive for developing a vaccine that can provide maximum efficacy with ease of delivery in as few doses as possible. It must be kept in mind that an adjuvant is not the active component in a vaccine and immunization; outcomes can vary greatly from one adjuvant to another when used in combination with the same vaccine antigen. Any given adjuvant-vaccine combination has to be evaluated on a case-by-case basis for safety, reactogenicity and efficacy in pre clinical trials. Ultimately, safety considerations outweigh any anticipated benefit and need to be evaluated for the development of a plan leading to human clinical trial [37].

Although aluminum compounds have been used for human vaccines since 1926, the need for developing new and more effective adjuvants has been felt increasingly for many other subunit and DNA vaccines, especially since the alum salts are relatively poor adjuvants [38]. Other experimental adjuvants used in a limited number of studies in humans include Quil A-derived saponin QS-21, bacterial and fungal derived moieties (e.g. muramyl dipeptide, monophosphoryl lipid A, CpG etc.), Water-in-oil emulsions (e.g., MF59, FIA, Montanide), particulate delivery systems (e.g., VLPs, liposomes, microspheres, ISCOM).

In certain preferred embodiments, the immunogenic compositions are formulated with an aluminum adjuvant. Aluminum based adjuvants are commonly used in the art and

include aluminum phosphate, aluminum hydroxide, aluminum hydroxy-phosphate, and amorphous aluminum hydroxyphosphate sulfate. Trade names of aluminum adjuvants in common use include ADJUPHOS, ALHYDROGEL, (both from Superfos Biosector a/s, DK-2950 Vedbaek, Denmark).

Non-aluminum adjuvants can also be used. Non-aluminum adjuvants include, but are not limited to, QS21, Lipid-A, Iscomatrix., and derivatives or variants thereof, Freund's complete or incomplete adjuvant, neutral liposomes, liposomes containing vaccine and cytokines or chemokines.

Emulsions of Montanide ISA 51 (a mineral oil adjuvant) and ISA 720 (oil-based non mineral oil) have been used in human clinical trials. A review of clinical trials (25 trials representing more than 4000 patients and 40,000 injections for Montanide ISA 51 and various trials representing 500 patients and 1500 injections for Montanide ISA 720) has revealed their general safety and strong adjuvant effect with mild to moderate local reactions.

In certain preferred embodiments of the invention, the method of the invention further comprises administering an adjuvant. In certain examples, the adjuvant is selected a water-in-oil emulsion. In other examples, the adjuvant is Aluminum hydroxide. However, any adjuvant that is suitable for administration with the immunogenic composition in the methods of the present invention can be suitably used.

Kits

Also included within the scope of the present invention are kits suitable for providing compositions of the immunogenic compositions as described herein.

For example, in such a kit one vial can comprise the immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens, and instructions for use in reducing transmission of *Plasmodium falciparum* or *Plasmodium vivax*.

In another example, a kit can preferably comprise one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens, and instructions for use in preventing malaria.

Preferably, the one or more pre-fertilization or post-fertilization antigens are selected from the group consisting of Pfs48/45, Pfs230, Pfs 28, Pfs25, Pvs48/45, Pvs 230, Pvs 28, and Pvs25. In exemplary embodiments, the one or more pre-fertilization or post-fertilization antigens is derived from a codon harmonized gene.

Preferably, the kit will contain instructions for using the composition. Such instructions can be in the form of printed, electronic, visual, and or audio instructions.

EXAMPLES

Example 1

Expression and Purification of Correctly Folded Pfs48/45

Generally speaking it is often difficult to express *P. falciparum* proteins in a heterologous expression system and such problems are largely due to high A/T content giving rise to changes in codon usage frequencies. It is now increasingly being realized that synonymous codon substitutions are not always silent and changes in the frequency of codon usage affects protein structure and function. Differences in synonymous codon usage between expression and natural hosts greatly affect expression and stability of proteins. These codon differences have even greater consequences if they

occur infrequently and within the domains that encode polypeptide regions of higher ordered structure. The presence of infrequently used codons is believed to cause ribosome pausing leading to incorrect folding and premature termination of protein during translation. An algorithm that takes into account known relationships between codon usage frequencies and secondary protein structure (designated codon harmonization algorithm) [25] has been developed to identify regions of slowly translated mRNA that are putatively associated with 'link/end' polypeptide segments contributing to higher ordered structures in a protein. Thus modification of those 'link/end' region codons and other codons to match the frequency in the expression host can counter non-natural ribosomal pausing and allow for co-translational folding, of proteins as synthesis continues [25]. By allowing the translation machinery to slow or pause at the same position in the transcript in the expression host as in the natural host (i.e. *Plasmodium*), the secondary structures in the nascent proteins are formed at comparable rates and thus lead to proper conformational folding and solubility of the expressed protein.

The native sequence of Pfs48/45 is represented by NCBI accession number AF356146, corresponding to SEQ ID NO: 7.

Here, the native sequence of Pfs 48/45 as represented by SEQ ID NO: 7, lacking N-terminal signal sequence (amino acid residues 1-27) and C-terminal anchor (amino acid residues 428-448) was converted to 'codon harmonized' sequence designed for expression in *E. coli*. The amino acid sequence reported under AF356146 differs from that of Z22145 (NF54) by C32Y, K33N, T72N, K253N and N254K substitutions. The A/T contents of Pfs48/45 sequence before and after codon harmonization were 75% and 56%, respectively. The codon harmonized Pfs48/45 sequence containing a sequence tag coding for 6x histidines at the 5' end was synthesized and cloned into the pET (K-) expression vector [19] and expressed in BL21 (DE3) cells [FIG. 1a]. Initial attempts to induce the cells with IPTG indicated that the expressed protein might negatively impact the growth of *E. coli*. To overcome the toxicity of expressed protein, the expression strategy was modified to slow down protein expression by growing the cells at 30° C. in Luria-Bertani growth medium containing 1% glucose and induction with 0.1 mM IPTG for 3 h, which resulted in highly efficient induction of protein in the cell lysate at 50 kDa [FIG. 1b, left panel, encircled]. The cell lysate when treated with β -ME to reduce the disulphide bonds in the protein, showed slower electrophoretic mobility of the induced Pfs48/45 protein [FIG. 1b, right panel, encircled]. A similar observation was made with the native form of the protein on the gametocyte surface [22,23].

Western blot analysis of cell lysates either untreated or after treatment with 0.5% sarcosyl revealed that the recombinant protein was insoluble in the absence of sarcosyl detergent (data not shown), and hence the treatment with ionic detergent was required to facilitate extraction of the protein from the pellet. To selectively enrich expressed Pfs48/45, the lysate was first treated with non-ionic detergent Tween-80 to remove any soluble bacterial proteins followed by treatment with 0.5% sarcosyl in PBS [FIG. 1c]. This sequential detergent extraction resulted in partial solubilization of the expressed protein which could be further purified using Ni²⁺-NTA-Agarose beads (QIAGEN) by elution using imidazole (1 M), yielding ~3 mg purified protein/g of wet cell pellet [FIG. 1d, e]. The yield of purified CH-rPfs48/45 in 7 independent purifications varied between 15 and 25 mg per liter of induced culture. The conformational characterization of the

expressed protein designated CH-rPfs48/45 was achieved using a mAb IIC5-B10 [22], which recognizes a conformational reduction-sensitive epitope in the parasite derived native Pfs48/45. The mAb recognized the non-reduced form of CH-rPfs48/45 yielding 3 immunoreactive bands at ~50 kDa of the gel [FIG. 1f, lane 1]. In addition to these monomeric forms, purified CH-rPfs48/45 protein preparations consistently revealed the presence of a higher molecular weight band at ~98 kDa, presumed to be a dimer. When a similar Western blot analysis was carried out with CH-rPfs48/45 after reduction prior to SDS-PAGE, the mAb rather unpredictably showed recognition of a single band around 65-68 kDa [FIG. 1f, lane 2]. Previous biosynthetic metabolic labeling studies had established that non-reduced Pfs48/45 immunoprecipitated by the mAb IIC5B10 (a doublet migrating in the region of 45 and 48 kDa) coalesces into a single protein band of ~68 kDa when analyzed by SDS-PAGE after reduction [23]. On the other hand, the same mAb recognized only the non-reduced form of native Pfs48/45 in the gametocytes and gametes in Western blot analysis [24]. While the reasons for the totally unexpected recognition of reduced form of CH-rPfs48/45 are not so obvious, a possible interpretation may be that the functional target epitope of blocking antibodies might be conformationally more stable in the CH-rPfs48/45, and therefore not affected by the reduction conditions employed.

Example 2

Evaluation of Functional Immunogenicity of CH-rPfs48/45 in Mice in Different Adjuvant Formulations

The immunogenicity of CH-rPfs48/45 was assessed in three different adjuvant formulations: CFA, water-in-oil emulsion using Montanide ISA-51 and Aluminium hydroxide. FIG. 2a shows the IgG titer 2 weeks after the 2nd boost in CFA group and 2 weeks after the 3rd boost in Alum and Montanide ISA-51 groups. This formulation resulted in the highest antibody titer ranging from 3×10^5 to $>1 \times 10^6$ [FIG. 2a, top panel]. However, all mice immunized with CH-rPfs48/45 in the other two adjuvant formulations also showed high antibody titer, the range being ~70,000 to more than 100,000 in both Montanide ISA-51 and Alum formulations [FIG. 2a, middle and bottom panels]. Sera for IgG isotype distribution was also tested and all four subtypes (IgG1, IgG2a, IgG2b, IgG3) were more or less equally represented in sera from mice immunized with CFA formulation, [FIG. 2b, top panel]. On the other hand, IgG1 and to a lesser extent IgG2 were the dominant subtype in Montanide ISA-51 formulation [FIG. 2b, middle panel]. The overwhelming presence of IgG1 and negligible amount of IgG3 were noticed in the Alum formulation [FIG. 2b, bottom panel].

Individual mouse sera were also tested for their ability to recognize the native Pfs48/45 protein in gametocyte extracts in both reduced and non-reduced form in Western blot analysis. Polyclonal antisera against CH-rPfs48/45 recognized a combination of reduction-sensitive as well as reduction-insensitive epitopes in Pfs48/45 in the parasites. Sera from mice immunized with CFA and ISA-51 formulations recognized the native non reduced protein (48/45 kDa), however, the sera from mice immunized with alum formulation revealed recognition of 48/45 kDa protein and also a protein ~98 kDa [FIG. 3a, left panel]. The reduced form of the native protein was recognized by all of the aforementioned sera at ~64 kDa [FIG. 3a, right panel]. As previously demonstrated [24] the mAb IIC5B10 reacted only with nonreduced native parasite

antigen. The ability of the anti-CH-rPfs48/45 sera to recognize the native form of the protein was further tested by live immunofluorescence assay (IFA) using live extracellular gametes. FIG. 3b shows an example of strong gamete surface reactivity typical of that observed for sera from mice immunized with CH-rPfs48/45 in all three adjuvant groups.

To assess the functionality of the immunized sera, they were tested for transmission blocking activity in mosquitoes in membrane feeding assays (MFA) [10]. All the mice sera in the three adjuvant groups showed a strong (>98% reduction in the number of oocysts) transmission blocking activity at 1:2 dilution [Table 1, shown below] as compared to corresponding pre-immune sera.

TABLE 1

Animal no.	Serum dilution		
	1:2	1:4	1:8
CFA			
1	100.0 (0/20)	89.6 (8/20)	76.8 (18/33)
2	97.5 (14/41)	88.0 (7/16)	79.3 (24/38)
3	98.3 (7/28)	90.2 (4/15)	72.6 (16/34)
4	94.3 (12/22)	90.5 (9/26)	83.5 (21/42)
5	98.0 (9/30)	93.9 (6/20)	84.6 (15/28)
ISA 51			
6	96.1 (5/15)	88.0 (16/23)	76.8 (12/19)
7	98.4 (2/12)	89.9 (11/20)	74.9 (10/17)
8	96.4 (7/18)	92.5 (12/22)	69.7 (16/22)
9	96.2 (7/15)	90.6 (15/25)	72.6 (11/18)
10	95.1 (10/20)	86.2 (10/17)	79.1 (12/19)
Alum			
11	93.5 (11/24)	86.2 (14/30)	54.9 (21/29)
12	95.3 (9/26)	78.6 (16/24)	58.3 (14/20)
13	96.6 (12/31)	89.9 (6/15)	55.4 (15/22)
14	94.5 (7/16)	89.2 (9/17)	70.5 (16/26)
15	95.2 (5/16)	91.6 (6/16)	61.4 (16/23)

Table 1 shows MFA with sera from mice immunized with CH-rPfs48/45 formulated in CFA, Montanide ISA-51 or Alum. Individual mouse sera were tested for transmission blocking activity with respect to corresponding pooled pre-immune sera. Data are represented as percent transmission blocking activity (reduction in the number of oocysts per mosquito midgut). Numbers within parenthesis represent total number of infected mosquitoes/total number of mosquitoes dissected for each feed.

Geometric mean oocyst numbers per midgut in the presence of pooled pre-immune sera of mice immunized in various adjuvant formulations and tested at different dilutions ranged between 10.4 and 16.7. The decrease in oocyst number/midgut by each immune sera was significant ($P < 0.02$, Mann-Whitney test).

The transmission blocking effect was dependent upon the antibody dose as revealed by a gradual decrease with increasing sera dilutions. Sera from mice immunized with CH-rPfs48/45 vaccine in CFA and Montanide ISA-51 formulations as compared to Alum appeared to be relatively more potent blockers as apparent from the stronger transmission blocking activities at 1:8 dilution of sera in MFA.

Example 3

Evaluation of Functional Immunogenicity of CH-rPfs48/45 in Non Human Primates (Olive Baboons)

Backed by strong functional immunogenicity of CH-rPfs48/45 in three different adjuvant formulations in

mice, the CH-rPfs48/45 vaccine was next evaluated in non-human primates (*Papio anubis*, Olive baboons). The vaccine trial in baboons was approved by the institutional and scientific review committee of the Institute of Primate Research with a protocol #19/10/2007.

A group of 5 baboons (ranging 7.6 to 12.2 kg in body weight) were immunized with 50 µg of CH-rPfs48/45 in Montanide ISA-51, water-in-oil emulsion. Our choice of the adjuvant for these studies was dictated, in part, by the fact that the adjuvant has already been in use as an investigational adjuvant in clinical trials in humans [25] and that the CH-rPfs48/45 vaccine formulated in Montanide ISA-51 was strongly effective in murine immunization studies (above). The vaccine, dose, route and schedules selected were based on experience with numerous other malaria vaccine trials in nonhuman primates [26,27,28]. Here, the vaccine was delivered through the intramuscular route (quadriceps, two sites) and boosted twice with the same dose of protein at 4 and 12 weeks post primary immunization [FIG. 4a].

To evaluate the immunogenicity of CH-rPfs48/45 in baboons, blood samples were collected from the immunized animals and assessed by ELISA. All, except one animal (Pan 3275), responded strongly after the primary immunization showing more than 8×10^4 IgG titers 1 month after primary immunization. The titers increased to >1 million, 1 month after the first dose of booster, even in the case of the single primary dose unresponsive animal [FIG. 4b], reaching an antibody titer of more than 2 million following the second booster injection. The IgG subtypes were also tested with various bleeds. Three out of five animals showed predominantly IgG1 compared to IgG2, and for the other two animals, the reverse was the case [FIG. 4c]. IgG3 was totally absent from immunized sera in all the bleeds tested.

Mosquito MFA showed strong transmission blocking activity by all animals for various bleeds obtained at different time points post immunization [Table 2, shown below].

TABLE 2

Animals	Bleeds [% transmission blocking * (infected/total mosquito)]				
	Dec. 10, 2007	Jan. 10, 2008	Feb. 21, 2008	Mar. 06, 2008	May 05, 2008
Pan 3104	92.8 (14/27)	95.7 (11/25)	98.7 (6/22)	97.7 (7/27)	98.8 (4/19)
Pan 3140	94.4 (14/21)	97.3 (12/28)	98.1 (6/24)	98.4 (7/23)	97.8 (7/18)
Pan 3163	98.1 (5/19)	98.5 (5/18)	97.3 (9/27)	97.3 (7/20)	97.4 (11/24)
Pan 3275	88.1 (12/20)	95.3 (11/25)	96.2 (10/28)	96.2 (9/23)	97.6 (5/22)
Pan 3313	91.8 (13/25)	97.0 (9/24)	94.7 (13/27)	97.8 (8/20)	95.3 (12/24)

*P < 0.0001 (Mann-Whitney test)

Table 2 shows transmission blocking activity of sera of different bleeds at various time points from baboons immunized with CH-rPfs48/45 in Montanide ISA-51. MFA was done with sera collected on Dec. 10, 2007 (1 mo post primary immunization), Jan. 10, 2008 (1 mo post 1st boost, Feb. 21, 2008 (15 d post 2nd boost), Mar. 6, 2008 (1 mo post 2nd boost), and May 5, 2008 (3 mo post 2nd boost). The geometric mean of oocyst numbers/midgut in the presence of individual pre-immune sera were 22.24 (Pan 3104), 18.43 (Pan 3140), 22.1 (Pan 3163), 6.31 (Pan 3275), and 19.7 (Pan 3313), respectively. Numbers within parenthesis represent total number of infected mosquitoes/total number of mosquitoes dissected for each feed.

Even after primary immunization, the sera (at 1:2 dilution) were capable of impressive transmission blocking with more than 93% average reduction in the number of oocysts in comparison to pre-immune sera of corresponding animal.

Pre-immune sera from each animal were used as a measure of 100% transmission for the corresponding test sera. Pre-immune serum from one animal (Pan 3275) exhibited ~30% reduced transmission when compared with the pre-immune sera of other four baboons. It is possible that natural infection by other *Plasmodium*-like parasites, such as *Enteropoides* and *Hepaticystis* spp might elicit partly cross-reactive and inhibitory activity. The average transmission blocking activity increased to greater than 97% in all the animals after a booster dose. In order to titrate the blocking effectiveness of immune sera from these animals, sera obtained one month after the second boost were further tested at various dilutions (1:4, 1:8, 1:16) in MFA. While exhibiting strong blocking activity at 1:4 and 1:8 dilutions, the sera at 1:16 dilution were still able to reduce transmission (ranging from 74% to 86%).

After the second booster dose, all five animals were bled at monthly intervals and sera analyzed for antibody titers by ELISA and functional transmission blocking activity in MFA. As shown in FIG. 5, high antibody titers and high transmission blocking activities were maintained for more than 7 months suggesting further long lasting nature of immune responses elicited by CH-rPfs48/45 in nonhuman primates.

The data reported herein shows for the very first time the expression of full length soluble recombinant Pfs48/45 protein in proper conformation with high yield following application of the codon harmonization approach for recoding gene sequences. The recombinant protein (designated CH-rPfs48/45) showed remarkable immunogenicity and functional activity, i.e. transmission blocking activity in mice immunized in the three adjuvant formulations tested. Further significance of the results is reflected by evidence from pre-clinical vaccine testing in nonhuman primates. The vaccine revealed potent immunogenicity and effective blocking activity even after a single immunization which became much stronger (approaching 100% reduction) after a booster immu-

nization. Ease of expression and purification of protein at high yields, conformational folding and strong immunogenicity of CH-rPfs48/45 in mice and non human primates provide a much needed rationale for moving ahead with the development of malaria TBV based on Pfs48/45 antigen. Mosquito infection (oocyst load and percent infected mosquitoes) in the presence of 4/5 pre-immune sera were comparable to those obtained with normal human serum negative control.

Expression of *P. falciparum* proteins in heterologous hosts is especially challenging due to high A/T content within protein coding genes (>80%). All previous attempts to express properly folded full length recombinant Pfs48/45 have remained unsuccessful. As reported herein, codon harmonization was used to express Pfs48/45 in *E. coli* in a functionally correct conformation. Another important consideration for the development of a transmission blocking vaccine is the

production of proteins in correctly folded conformation. The results herein report a robust expression of Pfs48/45 after codon harmonization of the coding sequence, the purified protein was found to be in correct conformation as revealed by Western blot analysis using monoclonal antibodies directed against reduction-sensitive conformational epitopes. The recombinant Pfs48/45 was recognized by the same antibodies even after treatment with reducing agents, suggesting that the target epitopes in the expressed proteins are stable and not susceptible to reduction.

Further development of a transmission blocking vaccine based on Pfs48/45 is supported by the observation that the purified protein exhibited very strong and longer lasting functional immunogenicity in baboons. The antibodies elicited by vaccination continued to effectively suppress, even 5-6 months after the last booster immunization, infectivity (both oocyst burden and percentage of mosquitoes infected) of *P. falciparum* gametocytes in *Anopheles* mosquitoes. Previous studies have shown that Pfs48/45 proteins normally expressed in the erythrocytic gametocyte stage of the parasite is also a target of partially effective natural immune response. It has been hypothesized that a vaccine induced response could be further boosted during natural infection and thus help in maintaining higher antibody levels in the vaccinated people.

Methods

The invention was performed with, but not limited to, the following methods and materials.

Cloning, Expression and Purification of CH-rPfs48/45

The codon harmonized sequence of Pfs48/45 containing 6× Histidines at N-terminal end was synthesized by Retrogen Inc. and cloned into the expression vector pET(K-) in *E. coli* BL21 (DE3) competent cells (Invitrogen Corp.). The cells containing pET(K-)Pfs48/45 were grown overnight at 30° C. in LB media containing 1% glucose and 50 µg/ml Kanamycin. The overnight culture was diluted 100 fold into 1 l culture in above mentioned media and grown at 30° C. with agitation until the OD600 of the culture reached 1.00. The cells were then induced with 0.1 mM of IPTG and grown for 3 h at 30° C. The cells were then harvested and centrifuged at 3800×g at 4° C. for 20 min. The pellet was kept at -80° C. for further processing. The frozen pellet was resuspended in 1×PBS (pH 7.4) at a pellet to buffer ratio of 1:10 (w/v) and was lysed by microfluidization (Model M110Y, Microfluidics). The lysate was centrifuged at 24000×g for 45 min at 4° C. The lysate pellet was resuspended in 1×PBS containing 1% Tween-80 (final v/v), extracted for 30 min at 22° C. and centrifuged at 24000×g for 30 min at 4° C. The pellet was resuspended in 1×PBS containing 0.5% Sodium Lauroyl Sarcosine (sarcosyl) (final v/v), extracted and centrifuged as before. The supernatant was then passed through Ni-NTA agarose column (QIAGEN) according to manufacturer's protocol. The protein was eluted as 1 ml fractions with 1 M imidazole in 1×PBS as elution buffer. The protein was dialyzed against 1×PBS (pH 7.4) containing 10% glycerol and 0.2% Tween-80. Finally, the protein content was estimated using BCA™ Protein Assay kit (Pierce) and the endotoxin level in the protein was measured using QCL-1000 Endpoint chromogenic LAL assay kit (Lonza).

Characterization of CH-rPfs48/45

The CH-rPfs48/45 was characterized by Western blot analysis. Briefly, samples from each purification step described above were run on SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad). The membrane was blocked overnight with 1×PBS containing 5% non-fat dry milk and 0.1% Tween-20 (blocking buffer) at 4° C. Following blocking, the membrane was washed with 1×PBS containing

0.1% Tween-20 (wash buffer) and incubated with either 6×His mAb (Clontech) at 1:1000 dilution [FIG. 1d, e] or IIC5B10 mAb (MR4) at 1:5000 dilution [FIG. 1f] for 1 h at 22° C. The membrane was washed 4× in wash buffer for 30 min at 22° C. and incubated with HRP-conjugated anti-mouse IgG mAb (GE Healthcare) at 1:10000 dilution in blocking buffer for 1 h at 22° C. This was followed by washing 4× with wash buffer and ECL Plus chemiluminescent substrate (GE Healthcare) was used as detection reagent.

10 Immunization of Mice

Groups (n=5) of female BALB/c mice were immunized with 10 µg of CH-rPfs48/45 emulsified in either Complete Freund's Adjuvant (CFA) (Sigma) or Montanide ISA-51 (SEPPIC) or mixed in aluminum hydroxide (Alhydrogel, Brenntag) adjuvant through the intraperitoneal route. The mice immunized in CFA were boosted at 4 week intervals twice with the same quantity of protein in incomplete Freund's adjuvant. Mice in the other adjuvant groups were boosted at 4 week intervals thrice in Montanide ISA-51 or Alum, respectively. Groups of control mice were immunized with adjuvant formulations only. Blood was collected on day 0 (Pre-immune sera) and 4 weeks after primary immunization and 2 weeks post each boost for analysis of anti-Pfs48/45 IgG titer.

25 Immunization of Olive Baboons (*Papio anubis*)

The vaccine trial in baboons was approved by the institutional and scientific review committee of the Institute of Primate Research with a protocol #Oct. 10, 2007. Because these animals were trapped from their wild habitats, they were quarantined for 3 months and screened for the presence of any worms and protozoan parasites and successfully treated appropriately, if found infected, prior to initiating vaccination. Moreover, animals were also screened by three intradermal tuberculin tests and found to be negative for mycobacterial infections. Detailed hematological tests were also administered on all five animals during their quarantine period, just prior to and at the termination of the vaccine trial, and were certified to be in excellent health at all time points with no observable trial-related effects. A group of five baboons (ranging 7.6 to 12.2 kg in body weight) were immunized with 50 µg of CH-rPfs48/45 in Montanide ISA-51, water-in-oil emulsion. The animals were sedated with ketamine (10 mg/kg) for immunization and blood collection as per the schedule described in FIG. 4a.

45 ELISA

To assess the immunogenicity of CH-rPfs48/45, ELISA was done. Briefly, Immulon-2 plates were coated with 1.5 µg/ml CH-rPfs48/45 in carbonate-bicarbonate buffer (pH 9.6) overnight at 4° C., blocked with 5% milk in PBS and incubated with various dilutions of sera at 37° C. for 1 h. The plates were washed 5 times in PBS-0.05% Tween-20 (PBST) followed by further incubation with 1:10000 dilution of horseradish peroxidase conjugated anti-mouse IgG antibody for 1 hour at 37° C. After washing in PBST, wells were developed using ABTS substrate (Kirkegaard & Perry Laboratories Inc.) for 20 min at 22° C. and read at 405 nm in the ELISA reader. Anti-Pfs48/45 whole IgG end point titers were calculated from the highest group mean reciprocal serum dilution greater than the mean plus 3 standard deviations OD reading of pooled pre-immune sera in each assay. For IgG subtype analysis, mouse sera were tested at a single 1:1000 dilution. Various isotype-specific secondary antibodies used were anti-mouse IgG1, IgG2a, IgG2b and IgG3 from Kirkegaard & Perry Laboratories Inc.

65 The ELISA with baboon sera was done following a similar protocol and endpoint titers were calculated using the same criteria. The secondary antibody was anti-human IgG1, IgG2,

and IgG3 conjugated to peroxidase (The Binding Site, Birmingham, UK) and used at 1:5000 dilution. Various sera were tested at 1:5000 dilution for IgG subclass analysis.

Parasite

Plasmodium falciparum NF54 parasites were maintained using normal red blood cells and normal human serum (O+ve blood group) as described [32]. Stage V gametocytes were used in live IFA studies and membrane feeding assays. To extract gametocytes for Western blot analysis, the gametocyte culture was centrifuged at 1000×g for 5 min at 22° C. The RBC was lysed with 0.15% saponin in PBS for 5 min at 22° C. The gametocytes were collected after centrifugation at 1800×g for 5 min and washed thrice in 1×PBS. The gametocytes were resuspended in 25 mM Tris-Cl (pH 7.5) containing 150 mM NaCl and 1× protease inhibitor cocktail and kept frozen at -70° C. till use.

Live IFA

The gametocytes were harvested from 19 day culture and gametes were produced by incubating the gametocytes in exflagellation buffer and purified by discontinuous Nycodenz gradient centrifugation as described previously [33]. Extracellular gametes were incubated at 4° C. with 1:100 to 1:1000 dilution of immune murine sera for 60 min. Parasites were washed 3 times with 1% BSA in PBS followed by incubation with FITC-anti mouse antisera (Alexa fluor 488), 1:500 dilution at 4° C. for 60 minutes. After washing, cells were examined by upright fluorescent Nikon E800 microscope (Japan) at 100× magnification.

Membrane Feeding Assay

To test the transmission blocking activity, the murine and baboon immune sera were mixed with cultured *P. falciparum* (NF54) stage V gametocytes, normal red blood cells and normal human sera (donor blood group: O+) and fed to *Anopheles gambiae* (starved for 5-6 hours) mosquitoes through water jacketed glass membrane (stretched parafilm) feeders [10,33] Briefly, washed human red blood cells and cultures containing stage V gametocytes were resuspended to 66% hematocrit and 0.3% gametocytemia in normal human serum and maintained throughout at 37° C. Fifty microliters of various test sera (appropriately diluted in normal human sera) were mixed with 150 µl of resuspended gametocyte mix and quickly added to individual membrane feeders placed on top of cups containing starved mosquitoes. The mosquitoes were allowed to engorge for 15 min. The unfed mosquitoes were separated within 1 h of feeding and blood fed mosquitoes (typically 25-30 per cup) were maintained in the insectary at 26° C. at 80% relative humidity. In certain cases the total number of blood fed mosquitoes was less than 25 due to poor feeding. Moreover, a small number of blood fed mosquitoes (less than 5%) did not survive 9-10 days incubation period prior to dissection. Midguts were dissected 9-10 days after blood meal for enumeration of oocysts after staining with 1% mercurochrome. Transmission blocking activity of individual sera was calculated as percentage of reduction in oocyst number per midgut with test sera in comparison with pooled pre-immune sera (pre-immune murine or baboon sera was taken as allowing 100% transmission).

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any

single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

REFERENCES

- (2008) WHO: World Malaria Report.
- Snow R W, Guerra C A, Noor A M, Myint H Y, Hay S I (2005) The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 434: 214-217.
- Greenwood B M, Fidock D A, Kyle D E, Kappe S H, Alonso P L, et al. (2008) Malaria: progress, perils, and prospects for eradication. *J Clin Invest* 118: 1266-1276.
- Gosling R D, Ghani A C, Deen J L, von Seidlein L, Greenwood B M, et al. (2008) Can changes in malaria transmission intensity explain prolonged protection and contribute to high protective efficacy of intermittent preventive treatment for malaria in infants? *Malar J* 7:54.
- Vekemans J, Ballou W R (2008) *Plasmodium falciparum* malaria vaccines in development. *Expert Rev Vaccines* 7: 223-240.
- Bejon P, Lusingu J, Olotu A, Leach A, Lievens M, et al. (2008) Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. *N Engl J Med* 359: 2521-2532.
- Abdulla S, Oberholzer R, Juma O, Kubhoja S, Machera F, et al. (2008) Safety and immunogenicity of RTS,S/AS02D malaria vaccine in infants. *N Engl J Med* 359: 2533-2544.
- Carter R, Mendis K N, Miller L H, Molineaux L, Saul A (2000) Malaria transmission-blocking vaccines—how can their development be supported? *Nat Med* 6: 241-244.
- Dimopoulos G, Kafatos F C, Waters A P, Sinden R E (2002) Malaria parasites and the *anopheles* mosquito. *Chem Immunol* 80: 27-49.
- Kumar N (2007) A vaccine to prevent transmission of human malaria: a long way to travel on a dusty and often bumpy road. *Curr Sci* 92: 1535-1544.
- Kaslow D C (2002) Transmission-blocking vaccines. *Chem Immunol* 80: 287-307.
- Wu Y, Ellis R D, Shaffer D, Fontes E, Malkin E M, et al. (2008) Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51. *PLoS ONE* 3: e2636.
- Gerloff D L, Creasey A, Maslau S, Carter R (2005) Structural models for the protein family characterized by gamete surface protein Pfs230 of *Plasmodium falciparum*. *Proc Natl Acad Sci USA* 102: 13598-13603.
- van Dijk M R, Janse C J, Thompson J, Waters A P, Braks J A, et al. (2001) A central role for P48/45 in malaria parasite male gamete fertility. *Cell* 104: 153-164.
- Roeffen W, Lensen T, Mulder B, Teelen K, Sauerwein R, et al. (1995) A comparison of transmission-blocking activity with reactivity in a *Plasmodium falciparum* 48/45-kD molecule-specific competition enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 52: 60-65.
- Outchkourov N S, Roeffen W, Kaan A, Jansen J, Luty A, et al. (2008) Correctly folded Pfs48/45 protein of *Plasmodium falciparum* elicits malaria transmission-blocking immunity in mice. *Proc Natl Acad Sci USA* 105: 4301-4305.

17. Komar A A, Lesnik T, Reiss C (1999) Synonymous codon substitutions affect ribosome traffic and protein folding during in vitro translation. FEBS Lett 462: 387-391.
18. Kimchi-Sarfaty C, Oh J M, Kim I W, Sauna Z E, Calcagno A M, et al. (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315: 525-528.
19. Angov E, Hillier C J, Kincaid R L, Lyon J A (2008) Heterologous protein expression is enhanced by harmonizing the codon usage frequencies of the target gene with those of the expression host. PLoS ONE 3: e2189.
20. Darko C A, Angov E, Collins W E, Bergmann-Leitner E S, Girouard A S, et al. (2005) The clinical-grade 42-kilodalton fragment of merozoite surface protein 1 of *Plasmodium falciparum* strain FVO expressed in *Escherichia coli* protects *Aotus nancymai* against challenge with homologous erythrocytic-stage parasites. Infect Immun 73: 287-297.
21. Hillier C J, Ware L A, Barbosa A, Angov E, Lyon J A, et al. (2005) Process development and analysis of liver-stage antigen 1, a preerythrocyte-stage protein-based vaccine for *Plasmodium falciparum*. Infect Immun 73: 2109-2115.
22. Rener J, Graves P M, Carter R, Williams J L, Burkot T R (1983) Target antigens of transmission-blocking immunity on gametes of *plasmodium falciparum*. J Exp Med 158: 976-981.
23. Kumar N, Carter R (1984) Biosynthesis of the target antigens of antibodies blocking transmission of *Plasmodium falciparum*. Mol Biochem Parasitol 13: 333-342.
24. Carter R, Graves P M, Keister D B, Quakyi I A (1990) Properties of epitopes of Pfs 48/45, a target of transmission blocking monoclonal antibodies, on gametes of different isolates of *Plasmodium falciparum*. Parasite Immunol 12: 587-603.
25. Peek L J, Middaugh C R, Berkland C (2008) Nanotechnology in vaccine delivery. Adv Drug Deliv Rev 60: 915-928.
26. Collins W E, Barnwell J W, Sullivan J S, Nace D, Williams T, et al. (2006) Assessment of transmission-blocking activity of candidate Pvs25 vaccine using gametocytes from chimpanzees. Am J Trop Med Hyg 74: 215-221.
27. Wu Y, Przysiecki C, Flanagan E, Bello-Irizarry S N, Ionescu R, et al. (2006) Sustained high-titer antibody

- responses induced by conjugating a malarial vaccine candidate to outer-membrane protein complex. Proc Natl Acad Sci USA 103: 18243-18248.
28. Stewart V A, McGrath S M, Walsh D S, Davis S, Hess A S, et al. (2006) Pre-clinical evaluation of new adjuvant formulations to improve the immunogenicity of the malaria vaccine RTS,S/AS02A. Vaccine 24: 6483-6492.
29. Penny M A, Maire N, Studer A, Schapira A, Smith T A (2008) What should vaccine developers ask? Simulation of the effectiveness of malaria vaccines. PLoS ONE 3: e3193.
30. Guerra C A, Gikandi P W, Tatem A J, Noor A M, Smith D L, et al. (2008) The limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. PLoS Med 5: e38.
31. Roberts L (2008) Infectious disease. New malaria plan called ambitious by some, unrealistic by others. Science 322: 26-27.
32. Ifediba T, Vanderberg J P (1981) Complete in vitro maturation of *Plasmodium falciparum* gametocytes. Nature 294: 364-366.
33. Quakyi I A, Carter R, Rener J, Kumar N, Good M F, et al. (1987) The 230-kDa gamete surface protein of *Plasmodium falciparum* is also a target for transmission-blocking antibodies. J Immunol 139: 4213-4217.
34. Aguilar, J. G., and Rodriguez, E. G. (2007) Vaccine adjuvants revisited. Vaccine 25, 3752-3762
35. Sesardic, D., and Dobbelaer, R. (2004) European union regulatory developments for new vaccine adjuvants and delivery systems. Vaccine 22, 2452-2456
36. Peek, L. J. et al., (2008) Nanotechnology in vaccine delivery. Advanced Drug Delivery Reviews.
37. Pink, J. R., and Kieny, M. P. (2004) 4th meeting on Novel Adjuvants Currently in/close to Human Clinical Testing World Health Organization—organisation Mondiale de la Sante Fondation Merieux, Annecy, France, 23-25, June 2003. Vaccine 22, 2097-2102
38. Lindblad, E. B. (2004) Aluminium adjuvants—in retrospect and prospect. Vaccine 22, 3658-3668.
39. Aucouturier, J., et al. (2006) The use of oil adjuvants in therapeutic vaccines. Vaccine 24, 44-45

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 16

<210> SEQ ID NO 1

<211> LENGTH: 400

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 1

Asn Asn Asp Phe Tyr Asn Pro Ser Ala Leu Asn Ser Glu Ile Ser Gly
1 5 10 15

Phe Ile Gly Tyr Lys Cys Asn Phe Ser Asn Glu Gly Val His Asn Leu
20 25 30

Lys Pro Asp Met Arg Glu Arg Arg Ser Ile Phe Cys Asn Ile His Ser
35 40 45

Tyr Phe Ile Tyr Asp Lys Ile Arg Leu Ile Ile Pro Lys Lys Ser Ser
50 55 60

Ser Pro Glu Phe Lys Ile Leu Pro Glu Lys Cys Phe Gln Lys Val Tyr

-continued

65				70						75				80	
Thr	Asp	Tyr	Glu	Asn	Arg	Val	Glu	Thr	Asp	Ile	Ser	Glu	Leu	Gly	Leu
				85					90					95	
Ile	Glu	Tyr	Glu	Ile	Glu	Glu	Asn	Asp	Thr	Asn	Pro	Asn	Tyr	Asn	Glu
			100					105					110		
Arg	Thr	Ile	Thr	Ile	Ser	Pro	Phe	Ser	Pro	Lys	Asp	Ile	Glu	Phe	Phe
		115					120					125			
Cys	Phe	Cys	Asp	Asn	Thr	Glu	Lys	Val	Ile	Ser	Ser	Ile	Glu	Gly	Arg
	130					135					140				
Ser	Ala	Met	Val	His	Val	Arg	Val	Leu	Lys	Tyr	Pro	His	Asn	Ile	Leu
145					150					155					160
Phe	Thr	Asn	Leu	Thr	Asn	Asp	Leu	Phe	Thr	Tyr	Leu	Pro	Lys	Thr	Tyr
				165					170					175	
Asn	Glu	Ser	Asn	Phe	Val	Ser	Asn	Val	Leu	Glu	Val	Glu	Leu	Asn	Asp
			180					185					190		
Gly	Glu	Leu	Phe	Val	Leu	Ala	Cys	Glu	Leu	Ile	Asn	Lys	Lys	Cys	Phe
		195					200					205			
Gln	Glu	Gly	Lys	Glu	Lys	Ala	Leu	Tyr	Lys	Ser	Asn	Lys	Ile	Ile	Tyr
	210					215					220				
His	Asn	Lys	Leu	Thr	Ile	Phe	Lys	Ala	Pro	Phe	Tyr	Val	Thr	Ser	Lys
225					230					235					240
Asp	Val	Asn	Thr	Glu	Cys	Thr	Cys	Lys	Phe	Lys	Asn	Asn	Asn	Tyr	Lys
			245						250					255	
Ile	Val	Leu	Lys	Pro	Lys	Tyr	Glu	Lys	Lys	Val	Ile	His	Gly	Cys	Asn
			260					265					270		
Phe	Ser	Ser	Asn	Val	Ser	Ser	Lys	His	Thr	Phe	Thr	Asp	Ser	Leu	Asp
			275				280					285			
Ile	Ser	Leu	Val	Asp	Asp	Ser	Ala	His	Ile	Ser	Cys	Asn	Val	His	Leu
	290					295					300				
Ser	Glu	Pro	Lys	Tyr	Asn	His	Leu	Val	Gly	Leu	Asn	Cys	Pro	Gly	Asp
305					310					315					320
Ile	Ile	Pro	Asp	Cys	Phe	Phe	Gln	Val	Tyr	Gln	Pro	Glu	Ser	Glu	Glu
				325						330				335	
Leu	Glu	Pro	Ser	Asn	Ile	Val	Tyr	Leu	Asp	Ser	Gln	Ile	Asn	Ile	Gly
			340					345					350		
Asp	Ile	Glu	Tyr	Tyr	Glu	Asp	Ala	Glu	Gly	Asp	Asp	Lys	Ile	Lys	Leu
		355					360					365			
Phe	Gly	Ile	Val	Gly	Ser	Ile	Pro	Lys	Thr	Thr	Ser	Phe	Thr	Cys	Ile
	370					375					380				
Cys	Lys	Lys	Asp	Lys	Lys	Ser	Ala	Tyr	Met	Thr	Val	Thr	Ile	Asp	Ser
385					390					395					400

<210> SEQ ID NO 2

<211> LENGTH: 1200

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 2

aataacgact tctacaacc atcggctctc aactctgaaa tcagcggctt catcggctac 60

aagtgcaact tcagcaacga aggcgttcac aacctgaagc cagacatgcg agaacgacgc 120

agcattttct gtaatataca ctcgtacttc atctacgaca agatccgtct gatcatccca 180

aaaaaagct cgagcccaga gttcaaatc ctgcctgaaa aatgcttcca gaaagtttac 240

-continued

```

actgactacg agaaccgtgt tgaaactgac atctcggaac tgggctgat tgaatacgaa 300
atcgaagaaa acgacaccaa tccaaactac aacgaacgca ccatcacgat cagcccattc 360
tctccaaaag atattgaatt cttctgcttc tgcgacaaca ctgaaaaggt tatcagctct 420
atcgaagggc gttctgctat ggttcacgta cgagttctga aataccaca caacattctg 480
ttcactaacc tgaccaacga cctcttcacc tacctcccta aaacctaca cgaaagcaac 540
ttcgtttcta acgttctgga agttgaactg aacgacggcg aactgttcgt tctggcttgc 600
gaactcatta acaaaaaatg cttccaggaa ggcaaagaaa aagccctgta caaatctaac 660
aaaatcattt accacaacaa gctcactata ttcaaagctc cattctacgt taccagcaaa 720
gacgttaaca ccgaatgcac ctgtaaattc aaaaacaaca actacaaaat cgttctgaaa 780
ccaaaatagc aaaaaaaagt catccatggc tgcaatttta gcagcaacgt atctagcaaa 840
cacactttca ccgactctct ggacattagc ctggttgacg actctgctca cattagctgc 900
aatgttcacc tcagegaacc aaaatacaac cacctcgttg gectgaactg cccaggcgac 960
attatcccag actgtttctt ccaggtttac cagccagaaa gcaagaact cgaaccatcg 1020
aatattgttt acctggacag ccagatcaac atcggcgaca ttgaatacta cgaagacgct 1080
gaaggcgacg acaaaattaa actgttcggc atcgttggtt ctatcccaa aacgaccagc 1140
ttcacttgca tctgcaagaa ggacaaaaaa tctgcttaca tgaccgttac tatcgactct 1200

```

<210> SEQ ID NO 3

<211> LENGTH: 185

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 3

```

Lys Tyr Asn Asn Ala Lys Val Thr Val Asp Thr Val Cys Lys Arg Gly
1           5           10           15
Phe Leu Ile Gln Met Ser Gly His Leu Glu Cys Lys Cys Glu Asn Asp
20           25           30
Leu Val Leu Val Asn Glu Glu Thr Cys Glu Glu Lys Val Leu Lys Cys
35           40           45
Asp Glu Lys Thr Val Asn Lys Pro Cys Gly Asp Phe Ser Lys Cys Ile
50           55           60
Lys Ile Asp Gly Asn Pro Val Ser Tyr Ala Cys Lys Cys Asn Leu Gly
65           70           75           80
Tyr Asp Met Val Asn Asn Val Cys Ile Pro Asn Glu Cys Lys Asn Val
85           90           95
Thr Cys Gly Asn Gly Lys Cys Ile Leu Asp Thr Ser Asn Pro Val Lys
100          105          110
Thr Gly Val Cys Ser Cys Asn Ile Gly Lys Val Pro Asn Val Gln Asp
115          120          125
Gln Asn Lys Cys Ser Lys Asp Gly Glu Thr Lys Cys Ser Leu Lys Cys
130          135          140
Leu Lys Glu Asn Glu Thr Cys Lys Ala Val Asp Gly Ile Tyr Lys Cys
145          150          155          160
Asp Cys Lys Asp Gly Phe Ile Ile Asp Asn Glu Ser Ser Ile Cys Thr
165          170          175
Ala Phe Ser Ala Tyr Asn Ile Leu Asn
180          185

```

-continued

<210> SEQ ID NO 4
 <211> LENGTH: 555
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 4

```

aagtataaca acgccaaagt tactgtcgac actgtatgta aacgtggttt cctgattcaa      60
atgagcggcc acctcgaatg caaatgCGaa aacgacctgg tcctggtaaa cgaagaaacc      120
tgCGaagaaa aagttttaa atgCGatgaa aagactgtaa acaaaccgtg cggtgacttc      180
tcgaaatgca ttaaattga cggtaaccct gttcttatg cttgcaaatg caacctcggT      240
tacgacatgg taaacaacgt ttgcattccg aacgaatgca agaacgtaac ttgCGgcaat      300
ggcaaatgca ttctggacac ctCGaaccca gttaaaactg gtgtttgttc ttgcaacatt      360
gggaaagttc ctaacgtaca ggaccagaac aaatgctcta aagacggtga aacgaaatgt      420
tctctgaaat gtctgaaaga aaacgaaacg tgcaaagctg ttgacggtat ttacaaatgc      480
gactgcaaag acggtttcat tattgacaac gaatCGagca tttgactgc tttctctgct      540
tacaacattc tgaac                                                    555
  
```

<210> SEQ ID NO 5
 <211> LENGTH: 450
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 5

```

Met Leu Lys Arg Gln Leu Ala Asn Leu Leu Val Leu Ser Leu Leu
 1           5           10           15
Arg Gly Ile Thr His Thr Gln Met Ala Lys Gly Glu Val Lys Tyr Val
          20           25           30
Pro Pro Glu Glu Leu Asn Lys Asp Val Ser Gly Phe Phe Gly Phe Lys
          35           40           45
Cys Asn Phe Ser Ser Lys Gly Val His Asn Leu Glu Pro Ile Leu Thr
          50           55           60
Glu Lys Arg Ser Leu Val Cys Ser Ile Tyr Ser Tyr Phe Ile Tyr Asp
65           70           75           80
Lys Ile Lys Leu Thr Ile Pro Lys Lys Ile Pro Gly Ser Lys Phe Lys
          85           90           95
Met Leu Pro Glu Lys Cys Phe Gln Thr Val Tyr Thr Asn Tyr Glu Lys
          100          105          110
Arg Thr Glu Glu Lys Ile Glu Asn Met Gly Leu Val Glu Tyr Glu Val
          115          120          125
Lys Glu Asp Asp Ser Asn Ser Glu Tyr Thr Glu Lys Ile Leu Thr Ile
          130          135          140
Ser Pro Phe Asn Thr Lys Asp Val Glu Phe Phe Cys Ile Cys Asp Asn
          145          150          155          160
Ser Glu Asn Val Ile Ser Asn Val Lys Gly Arg Val Ala Leu Val Gln
          165          170          175
Val Asn Val Leu Lys Tyr Pro His Lys Ile Thr Ser Ile Asn Leu Thr
          180          185          190
Lys Glu Pro Tyr Ser Tyr Leu Pro Asn Gln Val Asp Lys Thr Ser Phe
          195          200          205
  
```

-continued

Lys Ser His Lys Leu Asp Leu Glu Leu Gln Asp Gly Glu Leu Val Val
 210 215 220
 Leu Ala Cys Glu Lys Val Asp Asp Lys Cys Phe Lys Lys Gly Lys Asp
 225 230 235 240
 Thr Ser Pro Leu Ser Leu Tyr Lys Ser Lys Lys Ile Val Tyr His Lys
 245 250 255
 Asn Leu Ser Ile Phe Lys Ala Pro Val Tyr Val Lys Ser Ala Asp Val
 260 265 270
 Thr Ala Glu Cys Ser Cys Asn Val Asp Ser Thr Ile Tyr Thr Leu Ser
 275 280 285
 Leu Lys Pro Val Tyr Thr Lys Lys Leu Ile His Gly Cys Asn Phe Ser
 290 295 300
 Ser Asp Lys Ser Thr His Asn Phe Thr Asn His Val Asp Met Ala Glu
 305 310 315 320
 Leu Gly Glu Asn Ala Gln Ile Thr Cys Ser Ile Glu Leu Val Asp Thr
 325 330 335
 Ser Tyr Asn His Leu Ile Gly Met Ser Cys Pro Gly Glu Val Leu Pro
 340 345 350
 Glu Cys Phe Phe Gln Val Tyr Gln Arg Glu Ser Pro Glu Leu Glu Pro
 355 360 365
 Ser Lys Ile Val Tyr Leu Asp Ala Gln Leu Asn Ile Gly Asn Val Glu
 370 375 380
 Tyr Phe Glu Asp Ser Lys Gly Glu Asn Ile Val Lys Ile Phe Gly Leu
 385 390 395 400
 Val Gly Ser Ile Pro Lys Thr Thr Ser Phe Thr Cys Ile Cys Arg Lys
 405 410 415
 Gly Lys Lys Ile Gly Tyr Met Ser Val Lys Ile Ala Ala Gly Tyr Phe
 420 425 430
 Gly Phe Leu Ala Lys Ile Phe Ile Leu Leu Ile Val Leu Leu Leu Leu
 435 440 445
 Tyr Phe
 450

<210> SEQ ID NO 6
 <211> LENGTH: 1274
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polynucleotide

<400> SEQUENCE: 6

catatggcac accatcatca tcatcatccc ggggatccg gttctgttac catggccaag 60
 ggcgagggtca aatatgtgcc tccagaggag ctgaataagg atgtgagcgg cttttttggg 120
 ttttaagtga attttagcag caagggcgtc cataacctcg agcctattct gacggagaag 180
 cgaagcctgg tctgtagcat ttatagctat tttatztatg ataagattaa actgacgatt 240
 cctaaaaaaaa ttccaggctc gaagttaaag atgctcccag agaagtgttt tcaaacgggtg 300
 tatactaatt atgagaagcg cacggaggag aagattgaga atatgggcct cgtggagtat 360
 gaggtgaagg aggatgatag caactcggag tatacggaga agattctgac gattagcctt 420
 tttaacacga aagatgtgga gtttttttgt atttgtgata actcggagaa cgtcattagc 480
 aatgtgaaag gccgagtggc gctcgtgcaa gtgaatgtgc tcaagtatcc tcataagatt 540
 acgagcatta acctgacgaa agagccatat tcgtatctgc ctaatcaagt ggataaaacg 600

-continued

```

agctttaagt cgcataagct cgatctcgag ctccaagatg gcgagctcgt ggtgctcgcg 660
tgtgagaagg tggatgataa gtgttttaag aaaggcaaag atactagccc actgagcctc 720
tataaaagca agaagattgt gtatcataaa aacctcagca tttttaaggc gcctgtgtat 780
gtgaagagcg cggatgtgac ggcggagtgt agctgtaatg tggatagcac gatttatact 840
ctcagcctca agcctgtgta tacgaagaaa ctcatcctatg ggtgtaattt tagctcggat 900
aagagcacgc ataattttac taatcatgtg gatatggccg agctggggga gaatgcgcaa 960
attacgtgta gcattgagct cgtggatagc agctataatc atctcattgg catgagctgt 1020
cctggggagg tgctgcctga gtgttttttt caagtgtatc aacgagagag cccagagctc 1080
gagcctagca agattgtcta tctcgatgcc caactcaata ttggcaatgt ggagtatttt 1140
gaggatagca aaggcgagaa tattgtgaag atttttgggc tcgtgggcag cattcctaag 1200
acgacgagct ttacgtgtat ttgtcgaaaa gggaaaaaga ttgggtatat gagcgtgaag 1260
tgataagcgg ccgc 1274

```

```

<210> SEQ ID NO 7
<211> LENGTH: 1347
<212> TYPE: DNA
<213> ORGANISM: Plasmodium falciparum

```

```

<400> SEQUENCE: 7

```

```

atgatgttat atatttctgc gaaaaaggct caagttgctt tcatcttata tatagtattg 60
gtattaagaa taataagtgg aaacaatgat ttttataatc ctagcgcttt gaatagtgaa 120
atatctggat ttataggata taagtgtaat ttttcaaatg aagggtttca taatttaaag 180
ccagatatgc gtgaacgtag gtctatTTTT tgcaacatcc attcgtattt tatatatgat 240
aagataagat taataatacc taaaaaaagt tcgtcacctg agttttaaatt attacccgaa 300
aaatgttttc aaaaagtata tactgattat gagaatagag ttgaaactga tatatcggaa 360
ttaggtttaa ttgaatatga aatagaagaa aatgatacaa accctaatta taatgaaagg 420
acaataacta tatctccatt tagtccaaaa gacattgaat ttttttgttt ttgtgataat 480
actgaaaagg ttatatcaag tatagaaggg agaagtgcta tggtagatgt acgtgtatta 540
aaatatccac ataataTTTT atttactaat ttaacaaatg atctttttac atatttgccg 600
aaaacatata atgaatctaa ttttgtaagt aatgtattag aagtagaatt aaatgatgga 660
gaattatttg ttttagcttg tgaactaatt aataaaaaat gttttcaaga aggaaaagaa 720
aaagccttat ataaaagtaa taaaataatt tatcataata agttaactat ctttaaagct 780
ccattttatg ttacatcaaa agatgttaat acagaatgta catgcaaatt taaaaataat 840
aattataaaa tagttttaa accaaaatat gaaaaaaaag tcatacacgg atgtaacttc 900
tcttcaaatg ttagttctaa acatactttt acagatagtt tagatatttc tttagttgat 960
gatagtgcac atatttcatg taacgtacat ttgtctgaac caaaatataa tcatttggtg 1020
ggtttaaatt gtctgtgga tattatacca gattgctttt ttcaagtata tcaacctgaa 1080
tcagaagaac ttgaaccatc caacattggt tatttagatt cacaaataaa tataggagat 1140
attgaatatt atgaagatgc tgaaggagat gataaaatta aattatttgg tatagttgga 1200
agtataccaa aaacgacatc ttttacttgt atatgtaaga aggataaaaa aagtgcctat 1260
atgacagtta ctatagattc agcatattat ggatttttgg ctaaacatt tatattccta 1320
attgtagcaa tattattata tatttag 1347

```

```

<210> SEQ ID NO 8

```

-continued

```

<211> LENGTH: 620
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 8
catatggcac accatcatca tcatcatccc ggggatccg gttctggtac caagtataac      60
aacgccaaag ttactgtcga cactgtatgt aaacgtgggt tcttgattca aatgagcggc      120
cacctogaat gcaaatgcga aaacgacctg gtcttggtaa acgaagaaac ctgcaagaa      180
aaagttttaa aatgcgatga aaagactgta aacaaaccgt gcggtgactt ctgaaatgc      240
attaaaattg acggtaaccc tgtttcttat gcttgcaaat gcaacctcgg ttacgacatg      300
gtaaacacacg tttgcattcc gaacgaatgc aagaacgtaa cttgcggcaa tggcaaatgc      360
attctggaca cctcgaaccc agttaaact ggtgtttggt cttgcaacat tgggaaagtt      420
cctaacgtac aggaccagaa caaatgctct aaagacggtg aaacgaaatg ttctctgaaa      480
tgtctgaaag aaaacgaaac gtgcaaagct gttgacggta ttacaaatg cgactgcaaa      540
gacggtttca ttattgacaa cgaatcgagc atttgactg ctttctctgc ttacaacatt      600
ctgaactgat aagcggccgc                                     620

```

```

<210> SEQ ID NO 9
<211> LENGTH: 1265
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 9
catatggcac accatcatca tcatcatccc ggggatccg gttctggtac caataacgac      60
ttctacaacc catcggctct caactctgaa atcagcggct tcatcggcta caagtgcaac      120
ttcagcaacg aaggcgttca caacctgaag ccagacatgc gagaacgacg cagcattttc      180
tgtaatatac actcgtactt catctacgac aagatccgtc tgatcatccc aaaaaaagc      240
tcgagcccag agttcaaaat cctgcctgaa aaatgcttcc agaaagtta cactgactac      300
gagaaccgtg ttgaaactga catctcggaa ctgggcctga ttgaatacga aatcgaagaa      360
aacgacacca atccaaacta caacgaacgc accatcacga tcagcccatt ctctcaaaa      420
gatattgaat tcttctgctt ctgcgacaac actgaaaagg ttatcagctc tatcgaaggg      480
cgttctgcta tggttcacgt acgagttctg aaatacccac acaacattct gttcactaac      540
ctgaccaacg acctcttcac ctacctcct aaaacctaca acgaaagcaa cttcgtttct      600
aacgttctgg aagttgaact gaacgacggc gaactgttcg ttctggcttg cgaactcatt      660
aacaaaaaat gcttccagga aggcaaagaa aaagccctgt acaaatctaa caaaatcatt      720
taccacaaca agctcactat attcaaagct ccattctacg ttaccagcaa agacgttaac      780
accgaatgca cctgtaaatt caaaaacaac aactacaaaa tcgttctgaa accaaaatac      840
gaaaaaaaaag tcatccatgg ctgcaatfff agcagcaacg tatctagcaa acacactttc      900
accgactctc tggacattag cctgggtgac gactctgctc acattagctg caatgttcac      960
ctcagcgaac caaaatacaa ccacctcgtt ggctgaaact gcccaggcga cattatccca      1020
gactgtttct tccaggttta ccagccagaa agcgaagaac tcgaaccatc gaatattggt      1080
tacctggaca gccagatcaa catcggcgac attgaatact acgaagacgc tgaaggcgac      1140

```

-continued

```

gacaaaaatta aactgttcg catcgttggc tctatcccaa aaacgaccag cttcacttgc 1200
atctgcaaga aggacaaaaa atctgcttac atgaccgtta ctatcgactc ttgataagcg 1260
gccgc 1265

```

```

<210> SEQ ID NO 10
<211> LENGTH: 1699
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

```

```

<400> SEQUENCE: 10

```

```

catatggcac accatcatca tcatcatccc gggggatccg gttctggtac caaagaatat 60
gtttgcgctt caccgaccag ctgaaaccga ccgaatctgg cccaaaagtt aaaaaatgcg 120
aagttaaagt taacgagccg ctgatcaaag ttaaaatcat ctgcccgtg aaaggcagcg 180
ttgaaaaact gtacgacaac atcgaatacg ttccaaaaaa atcgccgtac gttgttctga 240
ccaaagagga aactaaactc aaggaaaaac tgtgtgcaaa actcatttac ggctgctga 300
tcagccctac ggttaatgaa aaggagaaca acttcaaaga aggcgttatt gaattcactc 360
tgctccagt ggttcataag gctaccgtgt tctacttcat ctgcgacaac agcaaaaccg 420
aagacgacaa taaaaaaggc aaccgtggga ttgttgaagt gtacgttgaa ccgtacggca 480
acaaaattaa cggctgcgct tttctcgacg aagacgaaga agaagaaaaa tacggcaacc 540
agattgaaga agacgaacac aacgagaaga tcaaaatgaa aaccttttc acgcaaaaca 600
tctacaaaaa aaacaacatc taccctgtgt acatgaaact gtactcgggc gacatcggcg 660
gcattctctt cccaagaac atcaaaagca ccacgtgctt cgaagagatg atccataca 720
acaaagaaat caaatggaac aaagaaaaca aatctctggg caatctggtt aacaacagcg 780
ttgtttacaa caaagagatg aacgctaaat acttcaacgt tcaatacgtt catattccaa 840
cctcttacia agacaccctg aacctgttct gctctattat cctgaaagaa gaggaatcta 900
acctgattag cactagctac ctggtttacg tttctattaa cgaagaactg aacttcagcc 960
tctttgactt ctacgaaagc ttcggtccaa tcaaaaaaac gatccaggtt gctcagaaga 1020
acgttaacaa caaagaacac gactacacct gcgacttcac ggacaaactg gacaaaacgg 1080
ttccaagcac tgctaacggg aagaaactgt tcatctgccg taagcacctg aaagaattcg 1140
acaccttcac gctgaaatgc aacgttaaca aaaccagta cccgaacata gagatcttcc 1200
caaaaacctt gaaagacaaa aaggaagttc tgaaactgga cctcgacatc cagtaccaga 1260
tgttctctaa attcttcaaa tttaacaccc aaaacgctaa gtacctgaac ctgtaccctg 1320
actacctgat tttcccgctc aaccacatcg gcaaaaaaga actgaaaaac aaccaacct 1380
acaaaaacca caaagacgtg aaatacttcg agcagagcag cgttctgagc cctctgagct 1440
cggctgattc tctggggaaa ctgctgaact tcttgacac tcaggagacg gtttgctca 1500
cggaaaagat cgttacctg aacctgtcta taaacgagct gggcagcgac aacaacacct 1560
tcagcgttac cttcaagtt cggccgtaca tcgacattaa ggaaccattc tacttcatgt 1620
tcggctgcaa caacaacaaa ggccaaggga acataggcat tgttgaactg ctgatcagca 1680
agcagtgata agcggccgc 1699

```

```

<210> SEQ ID NO 11
<211> LENGTH: 1964
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

```

-continued

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 11

```

catatggcac accatcatca tcatcatccc ggggatccg gttctggtac ccacgactac    60
acctgcgact tcacggacaa actggacaaa acggttccaa gcactgctaa cgggaagaaa    120
ctgttcatct gccgtaagca cctgaaagaa ttcgacacct tcacgctgaa atgcaacgtt    180
aacaaaacc agtaccgaa catagagatc ttcccaaaaa ccctgaaaga caaaaaggaa    240
gttctgaaac tggacctcga catccagtac cagatgttct ctaaattctt caaatttaac    300
accaaaacg ctaagtacct gaacctgtac ccgtactacc tgattttccc gttcaaccac    360
atcggcaaaa aagaactgaa aaacaacca acctacaaaa accacaaaga cgtgaaatac    420
ttcgagcaga gcagcgttct gagccctctg agctcggtg attctctggg gaaactgctg    480
aacttctgg aactcagga gacggtttgc ctgacggaaa agatccgta cctgaacctg    540
tctataaac agctgggcag cgacaacaac accttcagcg ttacctcca agttccgccc    600
tacatcgaca ttaaggaacc attctacttc atgttcggct gcaacaaca caaaggcgaa    660
gggaacatag gcattgttga actgctgatc agcaagcagg aagaaaagat taaaggctgc    720
aactttcacg aaagcaaac ggactacttt aacgaaaata ttagctctga caccacgaa    780
tgcacctcc acgcttacga aaacgacatc attggcttca actgcctgga aactactcac    840
ccaaacgagg ttgaggttga agttgaagac gctgaaatct acctccagcc agagaactgc    900
ttcaacaacg tttacaaagg cctcaacagc gttgacatta ctactatcct gaaaaacgct    960
cagacctaca acatcaaca caagaaaacc ccaacgttcc tgaaaattcc gcctgacaac   1020
ctgctggaag acgtcgaat ttcttgtcag tgcactatta aacaggttgt taaaaaatc   1080
aaagttatta tcacgaaaa cgacaccgtt ctgctgaaac gtgaagtgca gagcgagagc   1140
accctggacg acaaaatcta caaatgcgaa cacgaaaact tcattaacct gcgtgttaac   1200
aaaaccttgc acgaaaacgt tgaatacacc tgcaacatca aaatcgagaa ctttttcaac   1260
tacattcaga tcttctgccc ggccaagac ctcggcattt acaaaaacat ccagatgtac   1320
tacgacattg ttaaacgac ccgtgttccg cagttcaaaa aattcaaca cgaagaactg   1380
caciaactga ttccaaacag cgaaatgctg cacaaaacca aagaaatgct gattctgtac   1440
aacgaagaaa aagtggacct cctgcacttc tacgttttcc tgccgatcta catcaaagat   1500
atctacgaat ttaacatcgt ttgcgacaac agcaaaacca tgtggaaaa ccagctgggc   1560
ggcaaagtta tctaccacat tactgttagc aaacgtgagc aaaaagttaa aggctgcagc   1620
ttcgacaacg aacacgctca catgttctct tacaacaaaa ctaacgttaa aaactgcatt   1680
atcgacgcta aacaaaaga cctcatcggc tttgtttgcc ctagcggcac gctgaaactg   1740
accaactgct tcaaagacgc tatcgttcac accaacctga ccaacattaa cggcatcctc   1800
tacctgaaaa acaacctcgc taatttcacc tacaacacc agttcaacta catggaaatc   1860
ccggctctga tggacaacga catcagcttc aaatgcatct gcgttgacct gaaaaaaaaa   1920
aaatacaacg tcaaagccc gctgggccc tgataagcgg ccgc                               1964

```

<210> SEQ ID NO 12

<211> LENGTH: 2105

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

-continued

<400> SEQUENCE: 12

```

catatggcac accatcatca tcatcatccc ggggatccg gttctgttac caaccgtcac    60
gtttgcgact ttagcaaaaa caacctgatt gttccggaaa gcctgaaaaa aaaagaagag   120
ctcggcggca acccgggtaa cattcactgc tacgctctgc tgaaccact ggacaccctg   180
tacgttaaat gcccaaccag caaagacaac tacgaagctg ctaaagttaa taccagcgaa   240
aatgataacg aatacgagct gcaggttatc agcctgatag aaaaacgttt ccacaacttc   300
gagacgctgg aatcgaagaa accaggcaac ggcgacgttg ttgttcacaa cggcgttggt   360
gacactggcc cagttctgga caattctacc ttcgaaaaat acttcaaaaa catcaaaatc   420
aaaccggaca aattcttcga gaaagttatc aacgaatacg acgacactga agaagaaaaa   480
gacctggaat ctatcctgcc aggggttatt gtttctccaa tgaaagttct gaaaaaaaaag   540
gaccattca ccagctacgc tgctttcgtt gttccgccga ttgttcctaa agacctgcac   600
ttcaaagttg aatgcaacaa caccgaatac aaagacgaaa accagtacat ctctggctac   660
aacggcatca tccacattga catcagcaac tctaaccgca aaattaacgg ctgcgacttt   720
agcactaata actctagcat tctgacctcg tctgttaaac tggttaacgg cgaaactaaa   780
aactgcgaaa tcaacatcaa caacaacgaa gttttcggca taatctgcca caacgaaacc   840
aacctggacc cggaaaaatg cttccacgaa atctactcta aagacaacaa aactgttaaa   900
aaattccgag aagttatccc aaacatcgac atctttagcc tgcacaacag caacaagaaa   960
aaagttgctt acgctaaagt tccactggac tacattaaca aactgctgtt cagctgcagc  1020
tgcaaaaacca gccacactaa caccatcggc acgatgaaag ttactctcaa caaagacgaa  1080
aaagaagaag aagacttcaa aaccgctcag ggcattaaac acaacaacgt tcacctgtgc  1140
aactttttcg acaaccaga actgaccttc gacaacaaca aaatcgttct gtgcaaaata  1200
gacgctgaat tatttagcga agttattatc cagctgccga tcttcggcac caagaacggt  1260
gaagaaggcg ttcagaacga agaatacaaa aaattcagcc tgaaaccgag cctgggttttc  1320
gacgacaata acaacgacat taaagttatc ggcaagaaa aaaacgaagt tagcatttct  1380
ctggctctca aaggggttta cggcaaccga attttcaact tgcacaaaaa cggcaaaaaa  1440
ggcgaaggca tttctttctt catcccaccg atcaaacagg acaccgacct gaaattcatc  1500
attaacgaaa ccatcgacaa cagcaacatt aaacagcgtg gcctgatcta cattttcgtt  1560
cgcaaaaacg ttagcgaaaa cagcttcaaa ctgtgcgact ttaccaccgg ctcgactagc  1620
ctgatggaac tgaactctca ggttaaagaa aaaaagtgta ctgttaaaat taaaaaaggc  1680
gacattttcg gcctcaaatg cccaaaaggc ttcgctatct tcccgcaggc ttgcttctct  1740
aacgttctgc tggaatacta caaatctgac tacgaagact ctgaacacat taactactac  1800
attcacaag acaaaaaata caacctgaaa ccaaaaagcg ttattgaact gatggacgaa  1860
aacttccgtg aactgcagaa catccagcag tacaccgcca tcagcaacat taccgacgtg  1920
ctgcacttta aaaacttcaa cctgggcaac ctcccgtga acttcaaaaa ccaactacagc  1980
accgcttacg ctaaagttcc ggacacgttc aacagcatta ttaattttag ctgcaactgc  2040
tacaaccggg aaaaacacgt ttacggcact atgcaggttg agagcgacaa ctgataagcg  2100
gccgc                                             2105

```

<210> SEQ ID NO 13

<211> LENGTH: 2039

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 13

```

catatggcac accatcatca tcatcatccc ggggatccg gttctggtac caacgaacac    60
atctgcgact acgaaaaaaaa cgaaagctta atcagcaccg tgccaaacga caccaaaaaa   120
atccagaaat ctatatgcaa aattaacgct aaagctctgg acggttggtac cattaatgct   180
ccacacacca aaaacttcac gccaaaagac tacttcccaa acagcagcct gatcactaac   240
gacaaaaaaaa ttgtgattac ttctgacaag aaaaacttcg ttacttacat cgaccaaac   300
aaaaaaaaact tcagcctcaa agacatctac atccagtctt tctacggcgt tagcctcgac   360
cacctcaacc agatcaaaaa aatccacgaa gaatgggacg acggtcacct gttctacca   420
ccacacaacg ttctgcacaa cgttgttctc aacaaccaca tcgtcaatct gagcagcgt   480
ctggaaggcg tcctgttcat gaaaagcaaa gttactggcg acgaaaccgc taccaaaaaa   540
aatactaccg tcccactga cggcgttagc tctattctga ttccgcccga cgtaaggaa   600
gacatcacct tccacctctt ctgcgggaaa agcaccacca aaaaaccgaa taaaagaat   660
accagcctcg ctctcattca catccacatc agcagcaatc gtaacattat tcacggctgc   720
gactttctgt acctgaaaa ccagaccaac gacgctatct ctaacaaca caacaacagc   780
tacagcatct tcaccacaa caaaaacacc gagaacaacc tcatctgca catcagcctg   840
attccgaaaa ctgttatcgg cattaatgc ccaacaaaa aactgaacc gcagacctgc   900
ttcgacgaag tgtactacgt taaacaggaa gacgttccat cgaaaactat caccgctgac   960
aaatacaaca cttctctaa agataaaatc ggcaacatcc tgaaaaacgc tataagcatt  1020
aacaaccggg acgaaaagga caacacctac acttacctga tcctgccgga aaaattcgaa  1080
gaagaactga tagacacgaa aaaagtctg gcttgacact gcgacaaca atacatcatc  1140
cacatgaaaa tcgaaaaatc taccatggac aaaatcaaaa tcgacgaaaa aaaaaccatt  1200
ggcaaagaca tctgcaata cgacgttact actaaagttg ctacttgca aattattgac  1260
accattgact cgagcgttct gaaagaacac cacaccgttc actacagcat taccctgagc  1320
cgttgggaca aactcattat taaataccgg accaacgaga aaaccactt tgaaaacttc  1380
ttcgtaacc cattcaacct gaaagacaaa gttctgtaca actacaaca accgatcaac  1440
atcgaacaca tactgccggg cgccattacc accgacatct acgacacgcg taccaaaatt  1500
aaacagtaca tcctgcgtat tccgccgtac gttcacaag acatccactt tagcctggaa  1560
ttcaataact cgctctctct gaccaaacag aaccagaaca ttatttacgg caacggtgac  1620
aaaattttca ttcacatcaa ccagggctac aaagaaattc acggctgca ctttaccggc  1680
aaatactcgc acctgttcac ctacagcaaa aaaccactgc cgaacgacga cgacatctgc  1740
aacgttacta tcggcaacaa cacctttagc ggcttcgctt gtctgtcgca cttcgaactg  1800
aaaccgaaca attgttttag cagcgtttac gactacaacg aagccaaca agttaaaaa  1860
ctgtttgacc tctcgaccaa agttgaactg gatcacataa aacagaacac tagcggctac  1920
accctcagct acattatctt caacaagaa tcgaccaaac tcaaatttag ctgcacctgt  1980
agctcgaatt acagcaacta cactatccga ataaccttcg acccatgata agcggccgc  2039

```

<210> SEQ ID NO 14

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
6xHis tag

<400> SEQUENCE: 14

His His His His His His
1 5

<210> SEQ ID NO 15

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 15

Met Ala His His His His His His Pro Gly Gly Ser Gly Ser Gly Thr
1 5 10 15

Asn Asn Asp Phe
20

<210> SEQ ID NO 16

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Plasmodium falciparum

<400> SEQUENCE: 16

Thr Ile Asp Ser
1

What is claimed is:

1. A method for preparing a codon harmonized Pfs48/45 antigen sequence encoded by a pre-fertilization gene comprising:

Determining the frequency of codon usage of the pre-fertilization gene coding sequence, wherein the Pfs48/45 sequence corresponding to the nucleic acid sequence represented by SEQ ID NO: 7;

Substituting codons in the coding sequence of SEQ ID NO: 7 with codons of similar frequency from a host cell which code for the Pfs48/45 antigen, thereby preparing a codon harmonized Pfs48/45 antigen sequence wherein the codon harmonized Pfs48/45 sequence comprises the nucleotide sequence of SEQ ID NO: 2.

2. An isolated codon harmonized nucleotide sequence that comprises the nucleotide sequence of SEQ ID NO: 2 prepared according to the method of claim 1.

3. A vector comprising a codon harmonized Pfs48/45 sequence suitable for expression in a cell, wherein the codon harmonized Pfs48/45 sequence comprises the nucleotide sequence of SEQ ID NO: 2.

4. A cell expressing the vector of claim 3.

5. A kit comprising an immunogenic composition comprising one or more *Plasmodium falciparum* or *Plasmodium vivax* pre-fertilization or post-fertilization antigens, and instructions for use in reducing transmission of *Plasmodium falciparum* or *Plasmodium vivax*,

together with one or more of the isolated codon harmonized nucleotide sequence comprising SEQ ID NO 2 and/or the vector of claim 3.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 8,501,926 B2
APPLICATION NO. : 13/120784
DATED : August 6, 2013
INVENTOR(S) : Nirbhay Kumar and Evelina Angov

Page 1 of 1

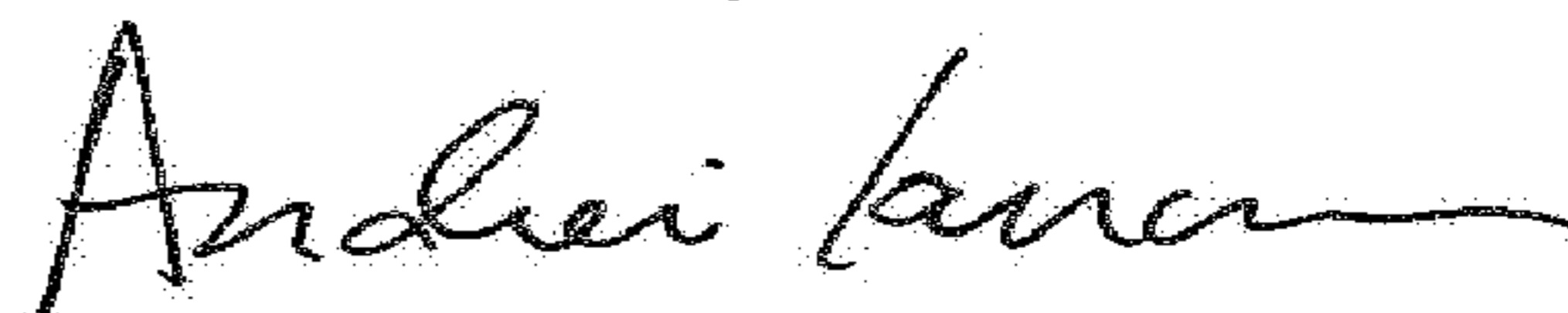
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1, immediately after the title, please add the following paragraph:

STATEMENT OF GOVERNMENTAL INTEREST

This invention was made with government support under grant number AI047089, awarded by the National Institutes of Health. The government has certain rights in the invention.

Signed and Sealed this
Twentieth Day of March, 2018



Andrei Iancu
Director of the United States Patent and Trademark Office